BENZIMIDAZOLES AND ANALOGS THEREOF AS ANTIVIRALS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority under 35 U.S.C. 119(e) from provisional application 60/430,495, filed 12/3/2003, the entire contents of said provisional application being expressly incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention relates to benzimidazole derivatives having antiviral activity, to compositions of matter comprising the same, and to antiviral methods of using the same. The invention also contemplates assays and diagnostic methods employing benzimidazole derivatives according to the invention. In some embodiments, there are provided anti-HCV benzimidazoles.

BACKGROUND

[0003] Hepatitis C virus (HCV) is a hepatoptropic, plus (+) strand RNA virus that presents a major threat to human health, infecting an estimated 170 million people worldwide. Acute HCV infection often leads to persistent infection, resulting in damage to the liver. Typical forms of liver damage caused by HCV include cirrhosis, chronic hepatitis and liver carcinoma. Less than 50% of patients respond to the current standard treatment, which is alpha interferon, alone or in combination with ribavirin. Accordingly, there has been intense interest in developing more efficacious anti-HCV drugs. It has been shown that the 5'-nontranslated region (5'-NTR) of the +RNA contains an internal ribosome entry site (IRES), which directs cap-independent initiation of virus translation. Furthermore, certain portions of the IRES element are essential for the HCV replication process. The IRES would appear to be a good target for antiviral compounds. Detailed descriptions of the HCV IRES and its functions have been presented, e.g. by Honda et al., in Journal of Virology, 73(2), 1165-74 (1999) (incorporated herein by reference, especially page 1166, Materials and Methods), and Kim, et al. in Biochem. Biophys. Res. Commun., 290, 105-112, (2002).

[0004] The activity of putative HCV IRES binding molecules can be measured using an HCV replicon per, e.g., the teaching of Lohmann et al. in Science, 285, 110-113 (1999) (incorporated herein by reference, especially page 111, Fig. 1 and legend thereof) and Yi, et al. in Virology, 304, 197-210 (2002).

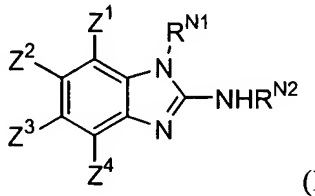
[0005] Given the high infection rate of HCV worldwide, and given the relatively low efficacy of the standard therapeutic methods, there is a need for anti-HCV compounds, e.g. for use in HCV assays and anti-HCV prophylactic and therapeutic applications.

SUMMARY OF THE INVENTION

[0006] The foregoing and further needs are met by embodiments of the present invention, which provide anti-HCV compounds, compositions and methods of use. In some embodiments, there are provided

compounds having anti-HCV activity as evinced by activity in the replicon assay as taught by Lemon et al., supra. In other embodiments, there are provided compositions comprising anti-HCV compounds, said compositions comprising an anti-HCV compound according to the present disclosure in admixture with one or more additives, e.g. diluents, excipients, adjuvants, etc. The present invention also provides methods of using anti-HCV compounds as described in more detail herein, said methods being directed toward attenuating expression of HCV RNA *in vitro*. The present invention also provides methods of using anti-HCV compounds as described in more detail herein, said methods being directed toward attenuating HCV *in vivo*. Some embodiments of the present invention provide compounds that inhibit HCV in the Lohman et al. replicon assay with IC_{50} values in the low micromolar concentrations.

[0007] The present invention provides compounds having antiviral, and in particular anti-HCV activity. The present invention also provides compositions containing compounds of the formula I:



(I),

wherein R^{N1} is a substituent of formula G^1-NX^1X^2, wherein G^1 is an optionally further substituted alkylene, which optionally forms, together with R^{N2}, a cyclo ring fused to the imidazolo ring of the benzimidazole, and each of X^1 and X^2 is independently H or an N-substituent, or X^1 and X^2 together form a heterocyclic ring, or X^1 together with G^1 forms a cyclic group and X^2 is H or an N-substituent; and each of Z^1, Z^2, Z^3 and Z^4 is H or a substituent, or two of Z^1, Z^2, Z^3 and Z^4 together form an optionally substituted ring, and further wherein at least one of Z^1, Z^2, Z^3 and Z^4 is other than H. In some embodiments, compounds and compositions according to the present invention demonstrate HCV replicon assay IC_{50} values in the low micromolar range.

[0008] The present invention further provides pharmaceutical compositions and methods of using the subject compounds as anti-HCV agents. In some embodiments, compounds of the invention may be used in HCV assays, in assays for measuring the relative efficacy of anti-HCV compounds or for treatment of HCV infection *in vivo*.

[0009] Methods for making the compounds of the invention are also disclosed. Other uses and advantages of embodiments of the present invention will be apparent to the person skilled in the art upon consideration of the disclosure, drawings and claims attached.

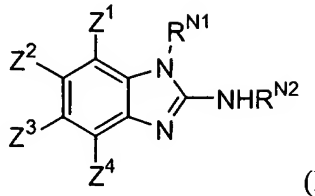
BRIEF DESCRIPTION OF THE FIGURES

[0010] Figure 1 is a bar graph of concentration of tested compounds in the presented tissues.

[0011] Figure 2 is a graph of plasma concentration over time for the compound having designation IBIS00528637, according to the depicted chemical structure. This graph further differentiates between routes of administration.

DETAILED DESCRIPTION OF THE INVENTION

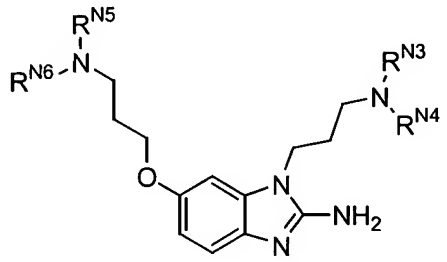
[0012] Provided are compounds of the formula:



(I),

wherein R^{N1} is a substituent of formula G^1-NX^1X^2, wherein G^1 is an optionally further substituted alkylene, which optionally forms, together with R^{N2}, a cyclic group, and each of X^1 and X^2 is independently H or an N-substituent, or X^1 and X^2 together form a heterocyclic ring, or X^1 together with G^1 forms a cyclic group and X^2 is H or an N-substituent; and each of Z^1, Z^2, Z^3 and Z^4 is H or a substituent, and wherein at least one of Z^1, Z^2, Z^3 and Z^4 is other than H.

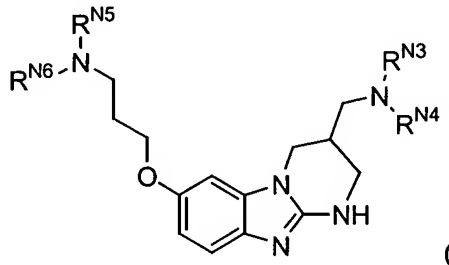
[0013] In some embodiments, the present invention provides compounds of formula A:



(A),

wherein each of R^{N3} is H or a substituent, R^{N4} is H or a substituent, or together R^{N3} and R^{R4} form a cyclic moiety that is optionally further substituted with one or more substituents; each of R^{N5} is H or a substituent, R^{N6} is H or a substituent, or together R^{N5} and R^{N6} form a cyclic moiety that is optionally further substituted with one or more substituents.

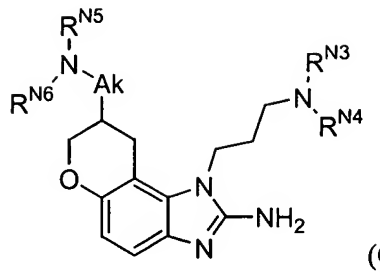
[0014] In some embodiments, the present invention provides compounds of formula B:



(B)

wherein each of R^{N3} is H, guanidine, amidines, substituted guanidines, amidines or a substituent, R^{N4} is H, guanidine, amidines, substituted guanidines, amidines or a substituent, or together R^{N3} and R^{R4} form a cyclic moiety that is optionally further substituted with one or more substituents; each of R^{N5} is H or a substituent, R^{N6} is H or a substituent, or together R^{N5} and R^{N6} form a cyclic moiety that is optionally further substituted with one or more substituents.

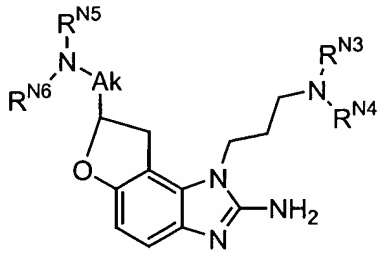
[0015] In some embodiments, the present invention provides compounds of formula C:



(C),

wherein Ak is C_1-C_6 alkylene, which is optionally further substituted; R^{N3} is H or a substituent, R^{N4} is H or a substituent, or together R^{N3} and R^{R4} form a cyclic moiety that is optionally further substituted with one or more substituents; each of R^{N5} is H or a substituent, R^{N6} is H or a substituent, or together R^{N5} and R^{N6} form a cyclic moiety that is optionally further substituted with one or more substituents.

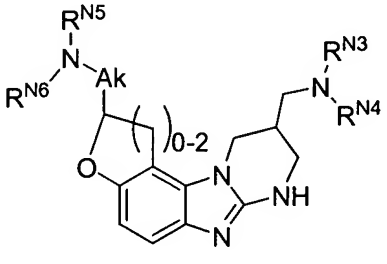
[0016] In some embodiments, the present invention provides compounds of formula D:



(D),

wherein Ak is C_1-C_6 alkylene, which is optionally further substituted; R^{N3} is H or a substituent, R^{N4} is H or a substituent, or together R^{N3} and R^{R4} form a cyclic moiety that is optionally further substituted with one or more substituents; each of R^{N5} is H or a substituent, R^{N6} is H or a substituent, or together R^{N5} and R^{N6} form a cyclic moiety that is optionally further substituted with one or more substituents.

[0017] In additional embodiments, the present invention provided compounds of the formula E:



(E),

wherein Ak is C_1-C_6 alkylene, which is optionally further substituted; R^{N3} is H or a substituent, R^{N4} is H or a substituent, or together R^{N3} and R^{R4} form a cyclic moiety that is optionally further substituted with one or more substituents; each of R^{N5} is H or a substituent, R^{N6} is H or a substituent, or together R^{N5} and R^{N6} form a cyclic moiety that is optionally further substituted with one or more substituents.

[0018] Especially advantageous embodiments of the present invention provide compounds of the foregoing formulae I and A-E having antiviral activity, and especially anti-HCV activity, as discussed in more detail herein. In some embodiments, the compounds are used *in vitro*, e.g. in assays, kits or some other milieu, as

test standards, e.g. for measuring the anti-HCV activity and/or potential of a candidate compound. In other embodiments, the compounds are used *in vivo*, e.g. as prophylactic or therapeutic compounds for the treatment of HCV infection, e.g. in humans.

[0019] In some embodiments, compounds of the foregoing formula in which, when X^1 or X^2 is further substituted alkyl, the further substituent does not comprise a 2-aminobenzimidazolyl moiety.

[0020] In further embodiments, there are provided compounds of the foregoing formula in which, when X^1 and X^2 form a ring, the ring is not a 2-aminobenzimidazol-1-yl ring.

[0021] In further embodiments, there are provided compounds of the foregoing formula in which neither Z^2 nor Z^3 is H, CF_3, unsubstituted C_1-C_2 alkyl, methoxy, ethoxy, or Cl.

[0022] In further embodiments, there are provided compounds of the foregoing formula in which Z^3 and Z^4 are not simultaneously methyl or Cl.

[0023] In further embodiments, there are provided compounds of the foregoing formula in which, when X^1 and X^2 are each alkyl (unsubstituted), Z^2 is neither methoxy nor N,N-dimethylaminopropyloxy.

[0024] In further embodiments, there are provided compounds of the foregoing formula in which, when X^1 and X^2 are each methyl or ethyl, Z^2 is not methoxy, ethoxy, or N,N-dialkylaminoalkyloxy.

[0025] Further preferred compound of the foregoing formula in which, when X^1 and X^2 are each methyl or ethyl, Z^2 is not neither methoxy nor N,N-dimethylaminopropyloxy.

[0026] In further embodiments, there are provided compounds of the foregoing formula in which, when X^1 and X^2 are each methyl, Z^3 is not aminomethyl or aminoethyl.

[0027] In further embodiments, there are provided compounds of the foregoing formula in which, when X^1 and X^2 are each methyl, Z^2 is not C_1-C_2 alkyl.

[0028] In further embodiments, there are provided compounds of the foregoing formula in which Z^2 and Z^3 are not simultaneously Cl.

[0029] It is to be understood that, when the compounds according to the present invention may be present either in their free base forms, as depicted in the formulae set forth herein, or as salts and/or hydrates thereof, and in particular as pharmaceutically acceptable salts thereof. Pharmaceutically acceptable salts are known in the art, as are hydrates, and the person having skill in the art will find it conventional to prepare such salts using art-recognized techniques. Exemplary salts include acid-addition salts, e.g. HCl, HBr, HI, HNO_3, H_3PO_4, NaH_2PO_4, Na_2HPO_4, H_3PO_3, NaH_2PO_3, Na_2HPO_4, H_2SO_4, $NaHSO_4$, carboxylic acids, such as acetic acid, malonic acid, capric acid, lauric acid, dichloroacetic acid, trichloroacetic acid, etc. Hydrates include hemihydrates, monohydrates, dihydrates, etc. Pharmaceutically acceptable salts include, HCl, H_2SO_4, acetic acid, malonic acid, capric acid, lauric acid, and other pharmacologically tolerated salts. Unless otherwise modified herein, the use of a free base formula is intended to include the salt and/or hydrate thereof.

[0030] As used herein, the term alkyl, unless otherwise modified, means an unsubstituted hydrocarbyl moiety. Acceptable alkyl groups include C_1-C_{12} alkyl, especially C_1-C_6 alkyl, e.g. methyl, ethyl, isopropyl, n-propyl, n-butyl, t-butyl, s-butyl. Accordingly, unless otherwise modified, the term alkyl includes, when appropriate, branched and unbranched alkyl moieties.

[0031] As used herein, the term alkenyl, unless otherwise further modified, means an unsubstituted hydrocarbyl moiety having at least one double-bond unsaturation in the hydrocarbyl moiety. Acceptable alkenyl moieties are C_2-C_{12}, especially C_2-C_6 alkenyl, e.g. ethenyl, prop-1-enyl, prop-2-enyl, etc. Accordingly, unless otherwise modified, the term alkenyl includes, where appropriate, branched and unbranched, mono- and poly-unsaturated alkenyl moieties.

[0032] As used herein, the term alkynyl, unless otherwise further modified, means an unsubstituted hydrocarbyl moiety having at least one triple-bond unsaturation in the hydrocarbyl moiety. Acceptable alkynyl moieties are C_2-C_{12}, especially C_2-C_6 alkynyl, e.g. ethynyl, prop-1-ynyl, prop-2-ynyl, etc. Accordingly, unless otherwise modified, the term alkenyl includes, where appropriate, branched and unbranched, mono- and poly-unsaturated alkenyl moieties.

[0033] As used herein, the term cyclyl means a substituent group having at least one cyclic ring structure. The term embraces both carbocyclyl and heterocyclyl. In turn, the term carbocyclyl means a cyclic structure having only carbon in the ring. Heterocyclyl, on the other hand, means a cyclic structure having both carbon and at least one non-carbon atom in the ring. As used herein, cyclyl, carbocyclyl, and heterocyclyl include, when not further modified, include mono- and polycyclic structures. Also, as used herein, cyclyl, carbocyclyl and heterocyclyl, connote ring structures that are unsubstituted only, whereas optionally further substituted cyclyl, carbocyclyl and heterocyclyl ring structures are identified by appropriate use of a suitable modifier.

[0034] The term carbocyclyl includes fully saturated, partially unsaturated and fully unsaturated ring structures. The term cycloalkyl is synonymous with a fully saturated carbocyclyl. Partially unsaturated cyclyoalkyl means a carbocyclyl group having at least one unsaturation, but not having the full complement of unsaturations possible within the ring structure. Fully unsaturated cycloalkyl means a carbocyclyl group having the full complement of unsaturations possible within the ring structure. Aryl means a carbocyclyl substituent having at least one ring that possesses aryl ring character. Exemplary cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, etc. Exemplary unsaturated cycloalkyl groups include cyclopentenyl, cyclohexenyl, cycloheptenyl, etc. Exemplary aryl groups include phenyl, naphthyl, 5,6,7,8-tetrahydronaphth-1-yl, etc.

[0035] The term heterocyclyl includes fully saturated, partially unsaturated and fully unsaturated ring structures. The term heterocyclyl also includes mon- and polycyclic ring structures in which at least one ring comprises carbon and at least one heteroatom in the ring. Exemplary heterocyclyl moieties have from one to three rings, comprise from one to about 14 carbons and from 1 to about 5 heteroatoms. Particular heterocyclyl moieties have from one to two rings and from one to about 10 carbons and from 1 to about 4

heteroatoms. Suitable heteroatoms include O, S and N. In particular embodiments, where necessary to satisfy its valence requirements, N may be unsubstituted (i.e. has an H to satisfy its valence of 3) or may be substituted with an alkyl or carbonyl, each of which may be further substituted. In particular embodiments, when such an N is substituted with alkyl, the alkyl is selected from methyl (Me) and ethyl (Et).

[0036] Fully saturated heterocyclyl includes pyrrolidinyl, piperidinyl, piperazinyl, N-alkyl piperazinyl, morpholino, N-alkylmorpholino, thiomorpholino, N-alkylthiomorpholino homopiperidinyl, homopiperazinyl, N-alkylhomopiperazinyl, homomorpholino, N-alkylhomomorpholino, homothiomorpholino, N-alkylhomothiomorpholino, tetrahydrofuranyl, tetrahydrothiophenyl, tetrahydrooxazolyl, N-alkyltetrahydrooxazolyl, tetrahydrothiazolyl, N-alkyltetrahydrothiazolyl, tetrahydroimidazolyl, N-alkyltetrahydroimidazolyl, etc.

[0037] Fully unsaturated heterocyclyl includes heteroaryl groups. Exemplary fully unsaturated heterocyclyl groups include pyrrolyl, imidazolyl, pyrenyl, pyridyl, pyrimidinyl, pyridazinyl, pyrazinyl, quinolyl, quoxalinyl, quinazolinyl, thiophenyl, furanyl, oxazolyl, thiazolyl, thiophenyl, pyranyl, thiopyranyl, benzofuranyl, indolyl, indazolyl, benzimidazolyl, benzothiazolyl, benzopyranyl, benzothiopyranyl, indazolyl, pyridopyrroly, etc. Partially unsaturated heterocyclyl includes partially unsaturated cognates of each of the following: pyrrolyl, imidazolyl, pyrenyl, pyridyl, pyrimidinyl, pyrazinyl, quinolyl, quoxalinyl, quinazolinyl, thiophenyl, furanyl, oxazolyl, thiazolyl, thiophenyl, pyranyl, thiopyranyl, benzofuranyl, indolyl, indazolyl, benzimidazolyl, benzothiazolyl, benzopyranyl, benzothiopyranyl, indazolyl, pyridopyrroly, etc.

[0038] Further substituents for cyclyl rings include halogens, e.g. Cl, Br and I, alkyl, alkenyl and alkynyl moieties, substituted alkyl, substituted alkenyl and substituted alkynyl moeities, wherein said further substituents are described herein. Further substituents for cyclyl rings also include further cyclyl rings, e.g. cycloalkyl, unsaturated cycloalkyl, fully unsaturated cycloalkyl, fully saturated heterocyclyl, partially unsaturated heterocyclyl, fully unsaturated heterocyclyl. Further substituents for cyclyl rings also include C(O)-R or C(O)O-R, wherein R is H, alkyl, or acyl, which alkyl may be further substituted. Further substituents for cyclyl rings also include NO_2, NH_2, NHR (where R is alkyl or may be further substituted), NR_2 (where R is alkyl or is further substituted), OSO_3H_2, SO_3H_2, OH, OR, wherein R is alkyl or acyl.

[0039] Further substituents for alkyl, alkenyl and alkynyl include halogens, e.g. Cl, Br and I, cyclyl rings, e.g. cycloalkyl, unsaturated cycloalkyl, fully unsaturated cycloalkyl, fully saturated heterocyclyl, partially unsaturated heterocyclyl, fully unsaturated heterocyclyl. Further substituents for alkyl, alkenyl and alkynyl also include C(O)-R or C(O)O-R, wherein R is H, alkyl, or acyl, which alkyl may be further substituted. Further substituents for cyclyl rings also include NO_2, NH_2, NHR (where R is alkyl or may be further substituted), NR_2 (where R is alkyl or is further substituted), OSO_3H_2, SO_3H_2, OH, OR, wherein R is alkyl or acyl.

[0040] Acyl groups include C(O)-R' groups, wherein R' is optionally substituted alkyl, alkenyl, alkynyl, cyclyl or O-R", wherein R" is H or R'. Especially suitable acyl groups include acetyl, benzoyl and t-butoxycarbonyl (BOC).

[0041] Alkylenyl means straight or branched divalent acyclic hydrocarbyl. Alkenylenyl means straight or branched divalent unsaturated hydrocarbyl, wherein at least one said unsaturations is a double bond. Alkynylenyl means straight or branced divalent acyclic hydrocarbyl having at least one triple bond unsaturation. Optionally further substituted alkylenyl, alkenylenyl or alkynenyl is an alkylenyl, alkenylenyl or alkynylenyl which has at least one additional substituent.

[0042] Replicon Assay: The Ntat2ANeo replicon containing cell line was obtained from Dr. S. Lemon at the University of Galvaston. Cells were grown, handled, treated with compound, and evaluated for HCV RNA levels as described previously (Yi, M.; Bodola, F.; Lemon, S. M. *Virology* **2002**, *304*, 197-210.) Briefly, the Ntat2ANeo cells were seeded into 96-well plates. The media was replaced 24 h later with fresh, G418-free media containing the indicated concentrations of drug. After the appropriate incubation period, cells were harvested, and quantitative RT-PCR assays were carried out using TaqMan chemistry on a PRISM 7700 instrument (ABI). For detection and quantitation of HCV RNA, primers complementary to the 5'-NTR region of HCV (Takeuchi, T., Katsume, A., Tanaka, T., Abe, A., Inoue, K., Tsukiyama-Kohara, K., Kawaguchi, R., Tanaka, S., and Kohara, M. *Gastroenterology* **1999**, *116*, 636–642.) were used. Results were normalized to the estimated total RNA content of the sample, as determined by the abundance of cellular GAPDH mRNA detected in a similar real-time RT-PCR assay using reagents provided with TaqMan GAPDH Control Reagents (Human) (Applied Biosystems).

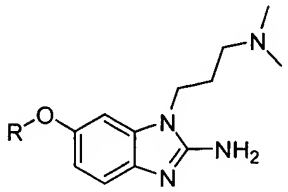
[0043] MTT Toxicity Assay: The MTT cell proliferation assay was used to test our compounds for cell toxicity (v van de Loosdrecht, A. A.; Beelen, R. H.; Ossenkoppele, G. J.; Broekhoven, M. G.; Langenhuijsen, M. M. *J. Immunol. Methods* **1994**, *174*, 311-320. The assay kit was purchased from American Type Culture Collection (Manassas, VA, USA), and treatment of cells and the specific assay protocol was carried out according to the manufacturer's recommendations. The MTT cell proliferation assay measures cell viability and growth by the reduction of tetrazolium salts. The yellow tetrazolium salt is reduced in metabolically active cells to form purple formazan crystals which are solubilized by the addition of detergent. The color was quantified by spectrophotometric means. For each cell type a linear relationship between cell number and absorbance is established, enabling quantification of changes of proliferation.

EXAMPLES

[0044] In the Examples 1-12 of 2-aminobenzimidazoles are illustrated in Scheme 1:

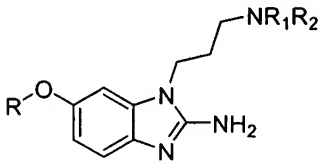
Scheme 1

Example 1
Alkylation methodology



R = alkyl

Example 2
Mitsunobu methodology



R = alkylamino
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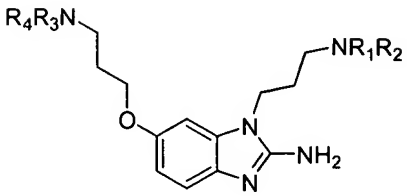
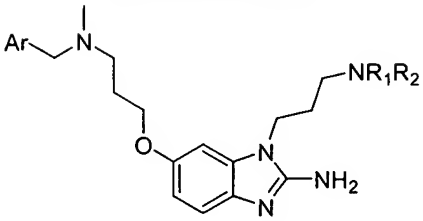
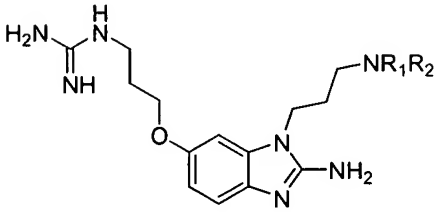
Example 3
Mesylate displacement



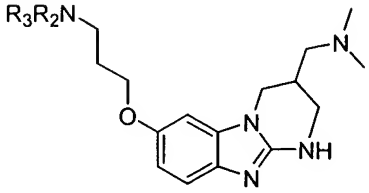
Example 4
Reductive Amination



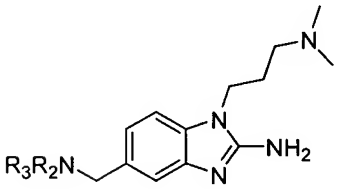
Example 5
Guanidine and mimetics



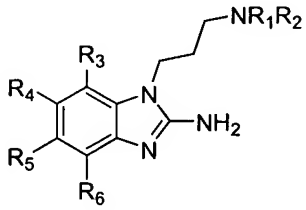
Example 6
Cyclic structures



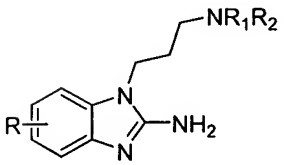
Example 7
Reductive Amination



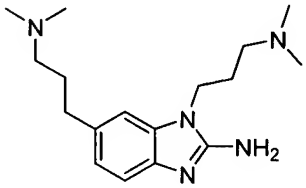
Example 8
Alkylation methodology



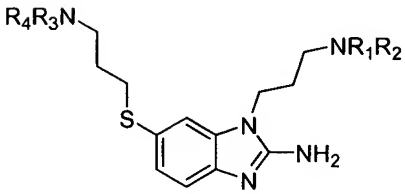
Example 9
SNAr methodology



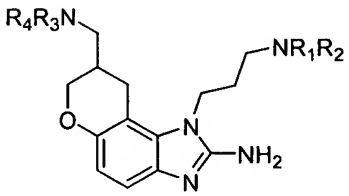
Example 10
Carbon tether analogs



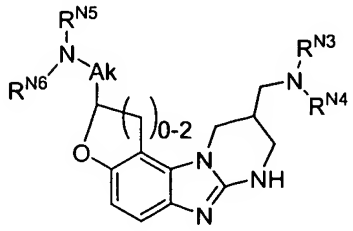
Example 11
C6 Sulfur analogs



Example 12
Constrained structures



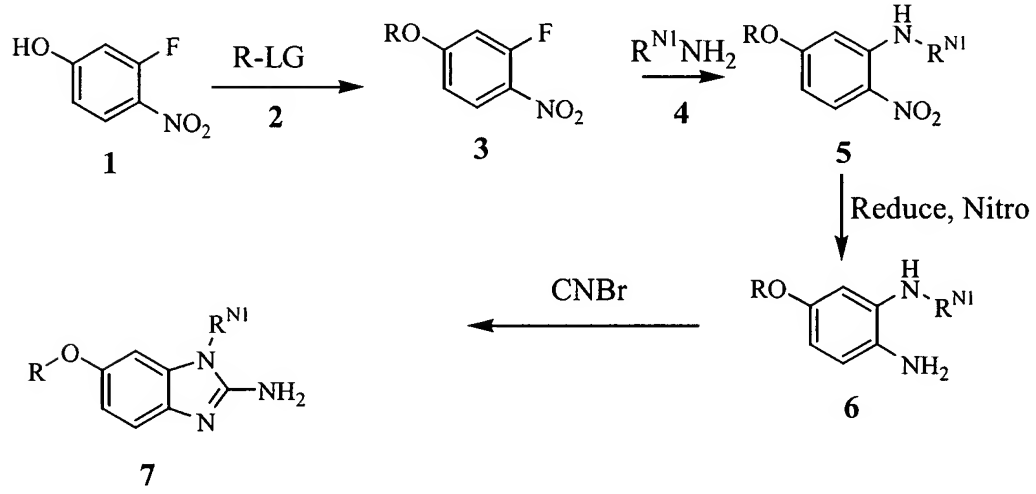
Example 15 and 16
Double Constrained Structures

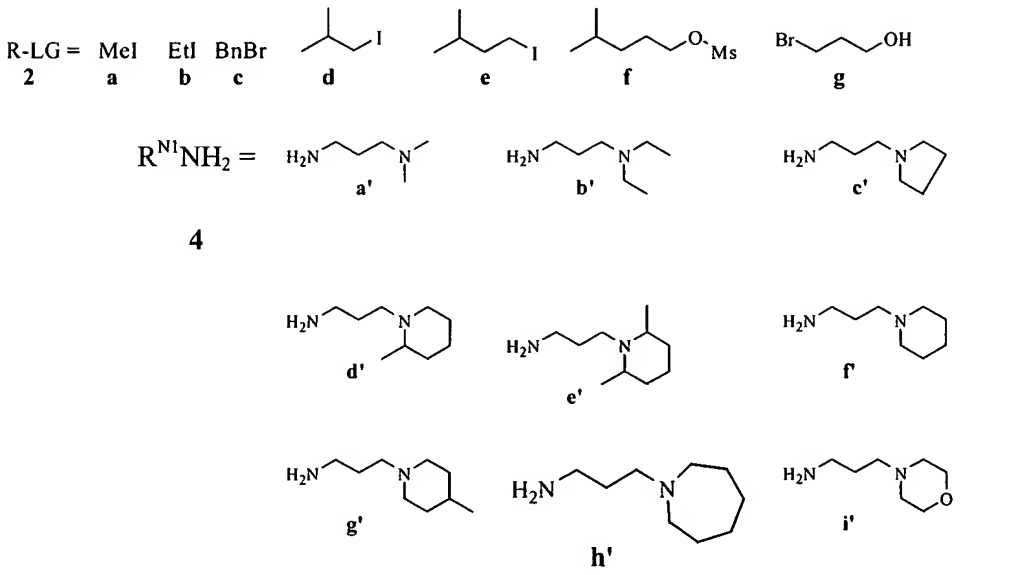


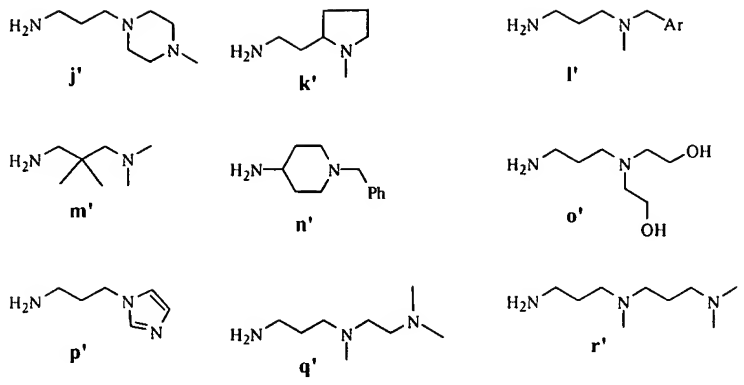
[0045] In each of the foregoing Examples 1-12 and 15 and 16 of Scheme 1, R, R^1, R^2, R^3, R^4, R^5, R^6 and R^7 are each, independently of the other, H or a substituent group. Exemplary substituent groups include alkyl, alkenyl, alkynyl, aryl, substituted alkyl, substituted alkenyl, substituted alkynyl or substituted aryl. Suitable alkyl groups include C_1-C_{12}, e.g. C_1-C_6 alkyl, such as methyl, ethyl, isopropyl, n-propyl, i-butyl, n-butyl, t-butyl, s-butyl, n-pentyl, etc. Suitable alkenyl groups include C_2-C_{12}, e.g. C_2-C_6 alkenyl, such as ethenyl, propen-3-yl, buten-4-yl, etc. Suitable alkynyl groups include C_2-C_{12}, e.g. C_2-C_6 alkynyl, such as ethynyl, prop-3-ynyl, etc. Suitable substituents include functional groups, such as NO_2, NH_2, COOH, halo, OH, $NH(C=NH)NH_2$, $NH(C=O)NH_2$, $CONH_2$, substituted guanidines, amidines, substituted amidines, etc. In each case where the functional group is an acid or a base group, the functional group may, together with a suitable counterion, form a salt, complex or chelate.

[0046] Example 1: Preparation of 6-alkoxy-2-amionbenzimidazoles:

Example 1







[0047] A mixture of 3-fluoro-4-nitrophenol **1** (3 mmol, 0.47 g), alkyl halide or alkylsulfonate **2** (3 mmol) and K_2CO_3 (3.3 mmol, 0.46g) in acetone (5 mL) was refluxed for 20 h. The reaction was then diluted with water and the aqueous layer was extracted with CH_2Cl_2 (twice). The combined organic layers were washed with water, brine, dried (over $MgSO_4$) and concentrated to provide **3**.

[0048] Crude **3** was then dissolved in toluene (6 mL) and treated with $R^{N1}NH_2$ **4** (3.3-6 mmol) and $CaCO_3$ (3.6 mmol, 0.36 g) and the reaction was refluxed for 2 h. The reaction was diluted with water and extracted with EtOAc (2X). The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated to provide **5**.

[0049] Crude **5** and 10% Pd/C (50 mg) were dissolved in EtOH (10 mL) and the mixture was hydrogenated at atmospheric pressure for 12 h at rt. The reaction was filtered through celite and concentrated to provide **6** as a dark oil.

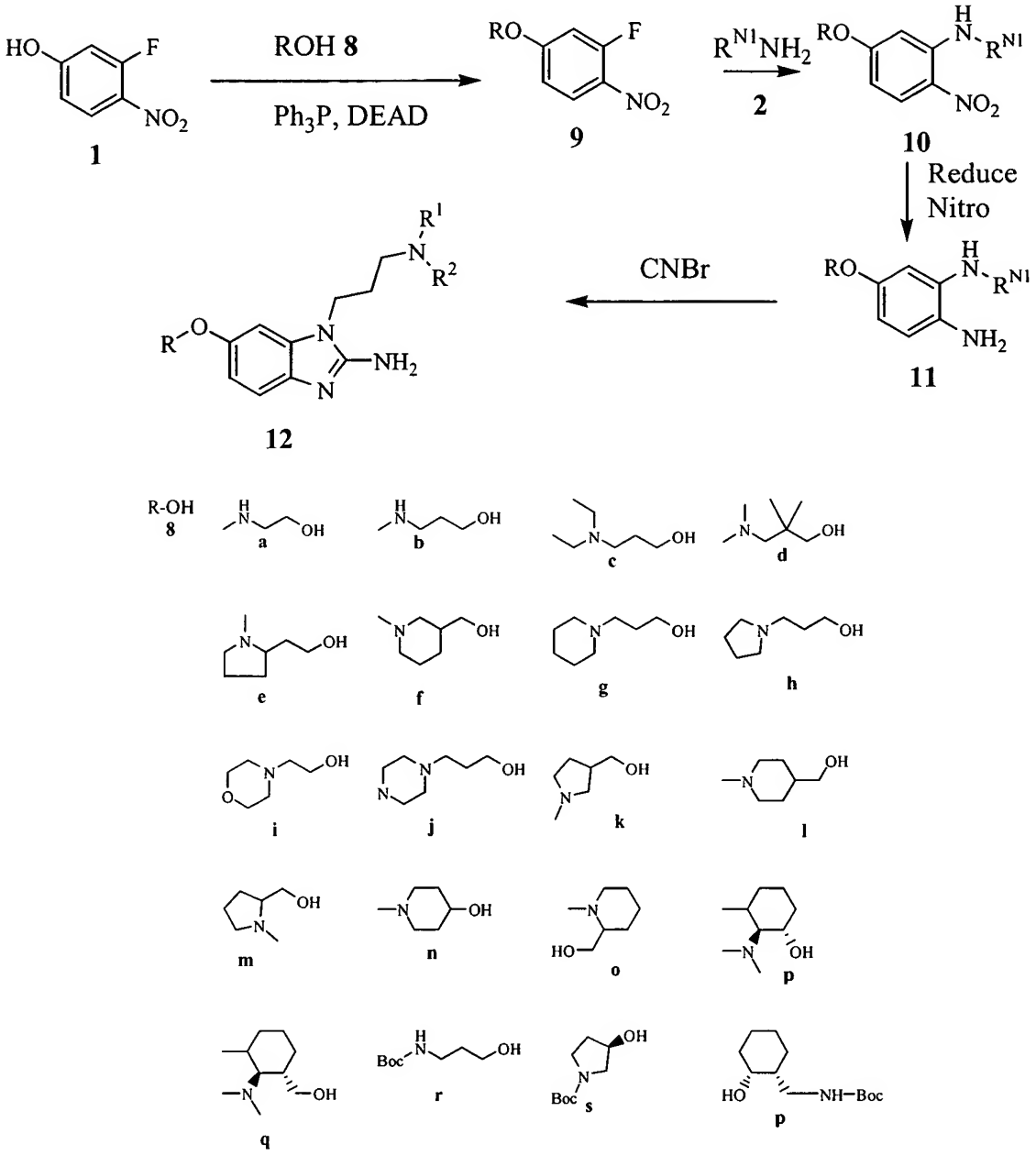
[0050] Crude **6** obtained above was dissolved in EtOH (3 mL) and treated with CNBr (3.3 mmol, 0.35 g). The reaction was stirred for 12 h, after which it was diluted with 4M NaOH until strongly basic (pH >12) and extracted with CH_2Cl_2 (3X). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated to provide **7** as a dark oil. Crude **7** could be purified by chromatography on aluminum oxide (activated, neutral, Brockmann 1, ~150 mesh) eluted with 5-10% MeOH/1% NH_4OH/CH_2Cl_2. Alternatively, crude **7** could also be purified by preparative reverse phase HPLC.

[0051] **1-(3-Dimethylaminopropyl)-6-methoxy-1H-benzoimidazol-2-ylamine (7aa')** was prepared according to the procedure described in Example 1 and purified by column chromatography to provide **7aa'**. LCMS: LC retention time 1.22 min; MS (ES^+) 249.2 (MH^+).

[0052] **3-[2-Amino-3-(3-dimethylaminopropyl)-3H-benzimidazoly-5-yloxy]-propan-1-ol (7ga')** was prepared according to the procedure described in Example 1 and purified by column chromatography to provide **7ga'**. LCMS: LC retention time 1.30 min.; MS (ES^+) 293.1 (MH^+).

[0053] **Example 2 – Synthesis of 6-alkylalkoxy-2-aminobenzimidazoles using Mitsunobu Alkylation**

Example 2



[0054] A mixture of 3-fluoro-4-nitrophenol **1** (8 mmol, 1.26 g), triphenyl phosphine (12 mmol, 3.14 g) and DIAD (12 mmol, 2.36 mL) in dry THF (70 mL) was cooled in an ice bath. Alcohol **8** (12 mmol) was added dropwise via a syringe to the above mixture and the reaction was stirred for 4h. The solvent was then removed by concentration under vacuum to provide a thick oil, which was dissolved in CH_2Cl_2 and sequentially extracted with 1M NaOH, water and 10% HCl. The acidic aqueous layer was separated and washed with CH_2Cl_2 and basified (pH >12) using solid NaOH and then further extracted with EtOAc. The EtOAc layer was then separated and washed with brine, dried and concentrated to provide crude **9** along with some (5-30%) DIAD byproduct.

[0055] Crude **9** obtained above was dissolved in toluene (20 mL) and treated with amine **4** (10-15 mmol) and K_2CO_3 (10 mmol, 1.38 g) and the reaction was refluxed for 2 h. The reaction was diluted with water and extracted with EtOAc (2X). The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated to provide **10**.

[0056] Crude **10** obtained above and 10%Pd/C (100-200 mg) were dissolved in EtOH (10 mL) and the mixture was hydrogenated at atmospheric pressure for 12 h at room temperature. The reaction was filtered through celite and concentrated to provide **11** as a dark oil.

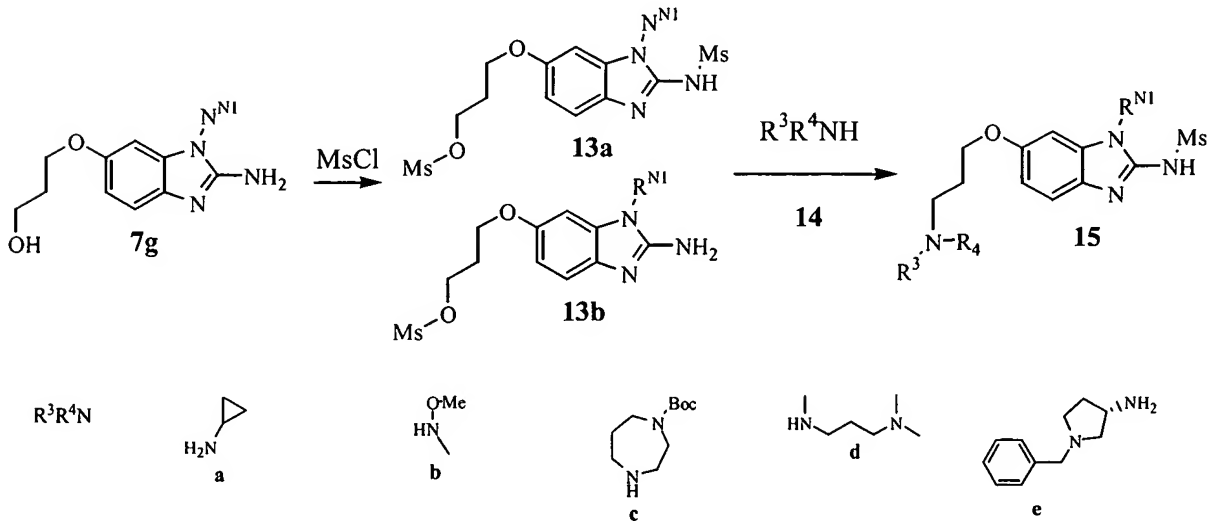
[0057] Crude **11** obtained above was dissolved in EtOH (10 mL) and treated with CNBr (10 mmol, 1.06 g). The reaction was stirred for 12 h, after which it was diluted with 4 M NaOH until strongly basic (pH > 12) and extracted with CH_2Cl_2 (3X). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated to provide **12** as a dark oil. Crude **12** could be purified by chromatography on aluminum oxide (activated, neutral, Brochmann 1, ~150 mesh), eluting with 5-10 % MeOH/1%NH_4OH/CH_2Cl_2. Alternatively, crude **12** could also be purified by preparative reverse phase HPLC.

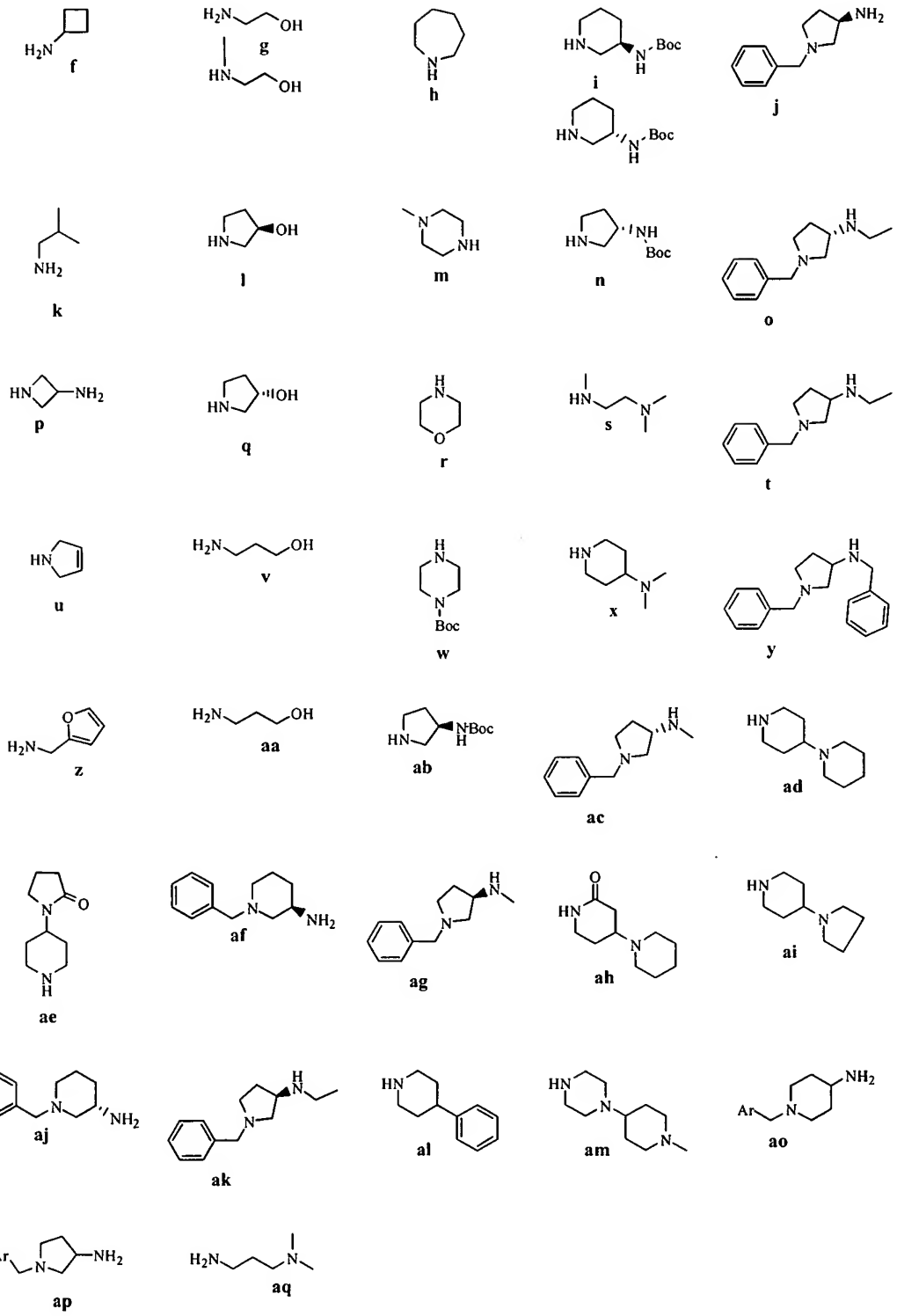
[0058] **1-(3-Dimethylaminopropyl)-6-(3-piperidin-1-ylpropoxy)-1H-benzimidazol-2-ylamine(12ga')** was prepared according to the procedure described in Example 2, and purified by peparative reverse phase HPLC to provide 159 mg of **12ga'·3TFA**. LCMS: LC retention time 0.96 min.; MS (ES^+) 348.1 (MH^+).

[0059] **1-(3-Dimethylaminopropyl)-6-(2-morpholin-4-ylethoxy)-1H-benzoimidazol-2-ylamine (12ia')** was prepared according to the procedure described in Example 2 and purified by preparative reverse-phase HPLC to provide 100 mg of **12ia'·3TFA**. LCMS: LC retention time 0.51 min.; MS (ES^+) 348.1 (MH^+).

[0060] **Example 3 – Synthesis of 6-alkylaminoalkoxy-2-aminobenzimidazoles by mesylate displacement**

Example 3





[0061] A mixture of 2-aminobenzimidazole **7g** (14.3 mmol, 4.3 g), 4-dimethylaminopyridine (1.4 mmol, 0.17 g) and K_2CO_3 (35.8 mmol, 4.94 g) in CH_2Cl_2 (15 mL) was cooled in an ice bath. Methanesulfonyl chloride (31.5 mmol, 2.43 mL) was added dropwise via a syringe to the above mixture and the reaction was stirred for an additional 3h. The reaction was then diluted with CH_2Cl_2 and the organic phase was washed with water, then with brine, then was dried over $MgSO_4$ and concentrated to provide a mixture of **13a** and **13b** in varying ratios. The crude mixture of **13a** and **13b** thus obtained was dissolved in DMF (40ML) and

this solution was distributed into 40 vials (1 mL each). K_2CO_3 (0.75 mmol., 0.103 g) and the desired primary or secondary amine (0.75 mmol – 0.88 mmol) was added to each reaction vessel. The individual reactions were heated at 60°C for 14 h, after which the contents of each vessel were diluted with CH_2Cl_2 and the organic layer was washed with 4M NaOH, washed with brine, then dried over $MgSO_4$ and concentrated to provide crude **15**, which was further purified by preparative reverse phase HPLC.

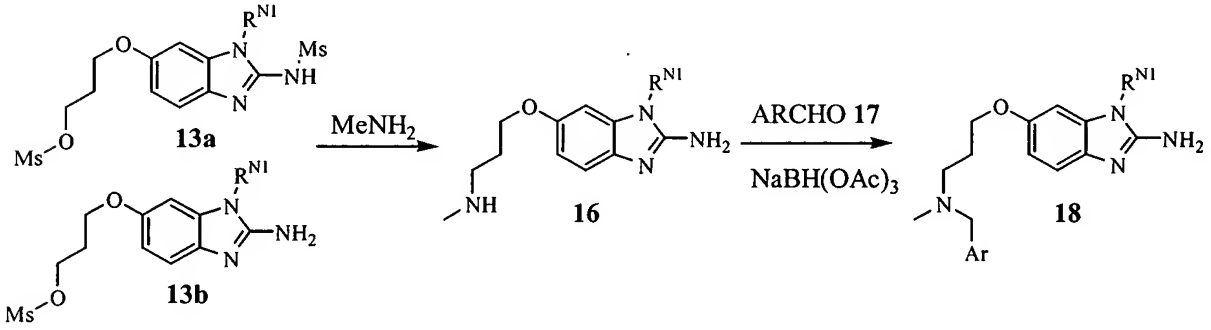
[0062] When the final product **15** contained a Boc protecting group, crude **15** was dissolved in a mixture of CH_2Cl_2/TFA (1:1, 2 mL). The reaction was stirred overnight and then concentrated, and the residue was purified by preparative reverse-phase HPLC (rpHPLC).

[0063] **N-{3-(2-Amino-3-(3-dimethylaminopropyl)-3H-benzimidazol-5-yloxy]propyl}-N,N',N'-trimethylpropane-1,3-diamine (15da')** was prepared according to the procedure described in Example 3 and purified by preparative rpHPLC to provide 68 mg of **15da'·3TFA.** LCMS: LC retention time 0.38 min.; MS (ES^+) 391.2 (MH^+).

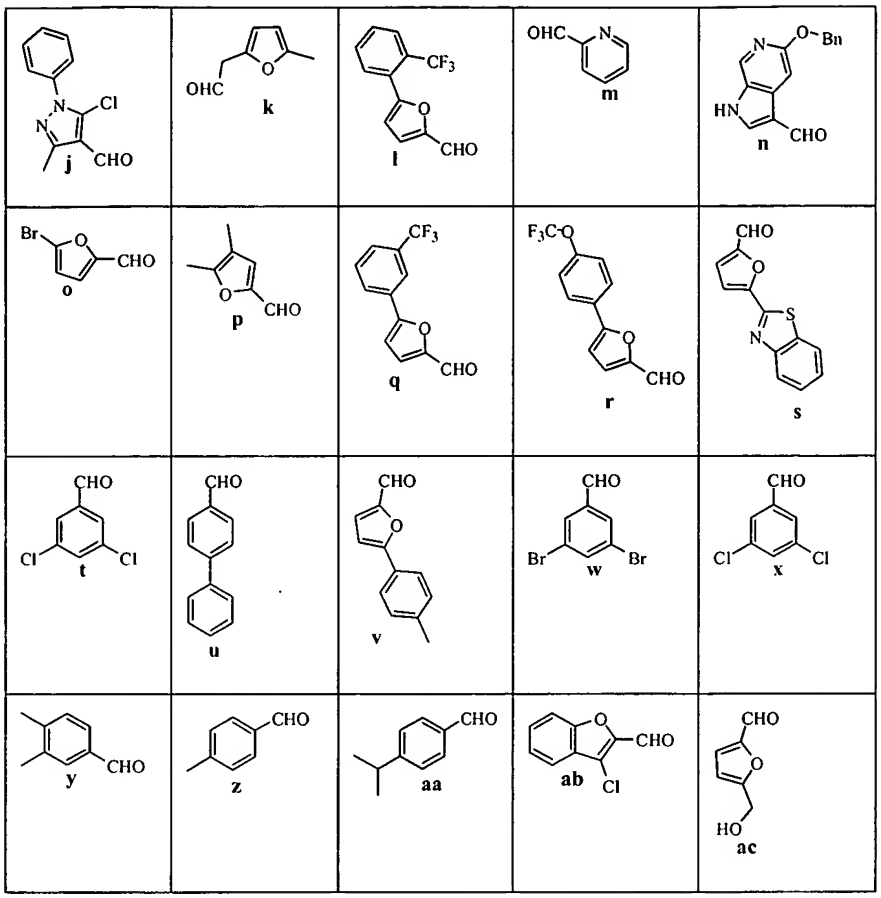
[0064] **N-{3-[2-Amino-3-(3-dimethylaminopropyl)-3H-benzoimidazol-5-yloxypropyl}-N,N',N'-trimethylethane-1,2-diamine (15sa')** was prepared according to the procedure described in Example 3 and purified by preparative rpHPLC to provide 81 mg of **15sa'·3TFA**. LCMS: LC retention time 0.40 min.; MS (ES^+) 377.2 (MH^+).

[0065] **Example 4 – Synthesis of 6-alkylaminoalkoxy-2-aminobenzimidazoles by reductive amination**

Example 4



ArCHO =				
	a	b	c	
e	f	g	h	i



[0066] A mixture of 2-aminobenzimidazole **7b** (7.15 mmol, 2.15 g), 4-dimethylaminopyridine (0.7 mmol, 0.085 g) and K_2CO_3 (18 mmol, 2.47 g) in CH_2Cl_2 (8 mL) was cooled in an ice bath. Methanesulfonyl chloride (14.3 mmol, 1.2 mL) was added dropwise via a syringe to the above mixture and the reaction was stirred for an additional 3 h. The reaction was then diluted with CH_2Cl_2 and the organic phase was washed with water, then with brine, and then dried over $MgSO_4$ and concentrated to provide a mixture of **13a** and **13b** in varying ratios. The crude mixture of **13a** and **13b** obtained above was then dissolved in DMF (31 mL) and treated with methylamine (15 mL of a 40% solution in H_2O) and heated at 60°C in a sealed vessel for 12 h. The reaction was cooled and diluted with saturated Na_2CO_3 and extracted with CH_2Cl_2 (3X). The combined organic layers were washed with brine, and then dried over $MgSO_4$ and concentrated to provide crude 2-aminobenzimidazole **16** as a dark, red oil (1 g).

[0067] Proton data for **16a'**: ^{1}H NMR (200 MHz, $CDCl_3$) d 7.24 (d, 1H), 6.7 (dd, 1H), 6.6 (d, 1H), 5.8 (s, br, 2H), 4.05 (t, 2H), 4.0 (m, 2H), 2.8 (t, 2H), 2.42 (s, 3H), 2.2 (s, 6H), 2.1 (m, 2H), 1.95 (m, 4H). LCMS: LC retention time 0.44 min.; MS (ES^+) 306.2 (MH^+).

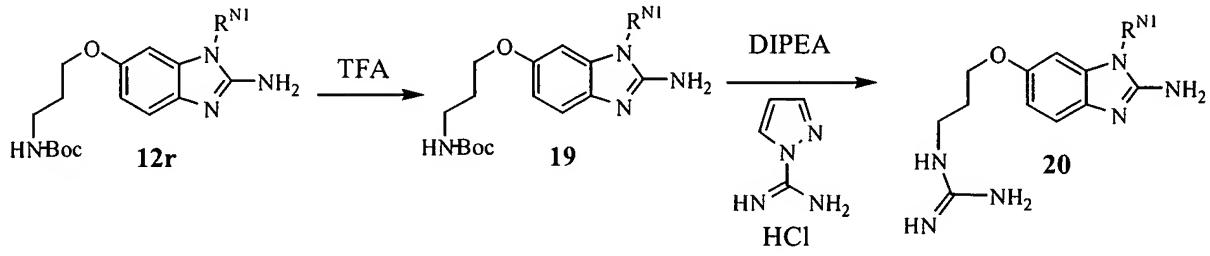
[0068] Crude 2-aminobenzimidazole **16** obtained above (0.33 mmol, 0.1 g) and the desired aldehyde **17** (0.33 mmol) was dissolved in dry CH_2Cl_2 (2 mL). Glacial acetic acid (1 drop) was added to the reaction , which was stirred for 5-10 min., followed by addition of sodium triacetoxyborohydride (0.66 mmol, 0.14 g). The mixture was stirred at room temperature for 12 h, after which it was diluted with CH_2Cl_2 and the organic phase was washed with saturated Na_2CO_3, then with brine, and then was dried over $MgSO_4$ and concentrated. Crude 2-aminobenzimidazole **18** thus obtained was further purified by preparative rpHPLC.

[0069] **6-[3-(Methyl-pyridin-2-ylmethylamino)-propoxy]-1-(3-pyrrolidin-1-ylpropyl)-1H-benzimidazol-2-ylamine (18mc')** was prepared according to the procedure described in Example 4 and purified by preparative reverse phase HPLC to provide 30 mg of **18mc'·3TFA**. LCMS: LC retention time 1.46 min. MS (ES^+) 423.1 (MH^+).

[0070] **1-[2-Methyl-piperidin-1-yl)propyl]-6-{3-[methyl-1H-pyrrol-2-ylmethyl)-amino]-propoxy}-1H-benzimidazol-2-ylamine (18ed')** was prepared according to the procedure described in Example 4 and purified by preparative rpHPLC to provide 73 mg of **18ed'·3TFA**. LCMS: LC retention time 1.63 min.; MS (ES^+) 439.2 (MH^+).

[0071] **Example 5 – Synthesis of 6-guanidinoalkoxy-2-aminobenzimidazoles**

[0072] **Example 5**



[0073]

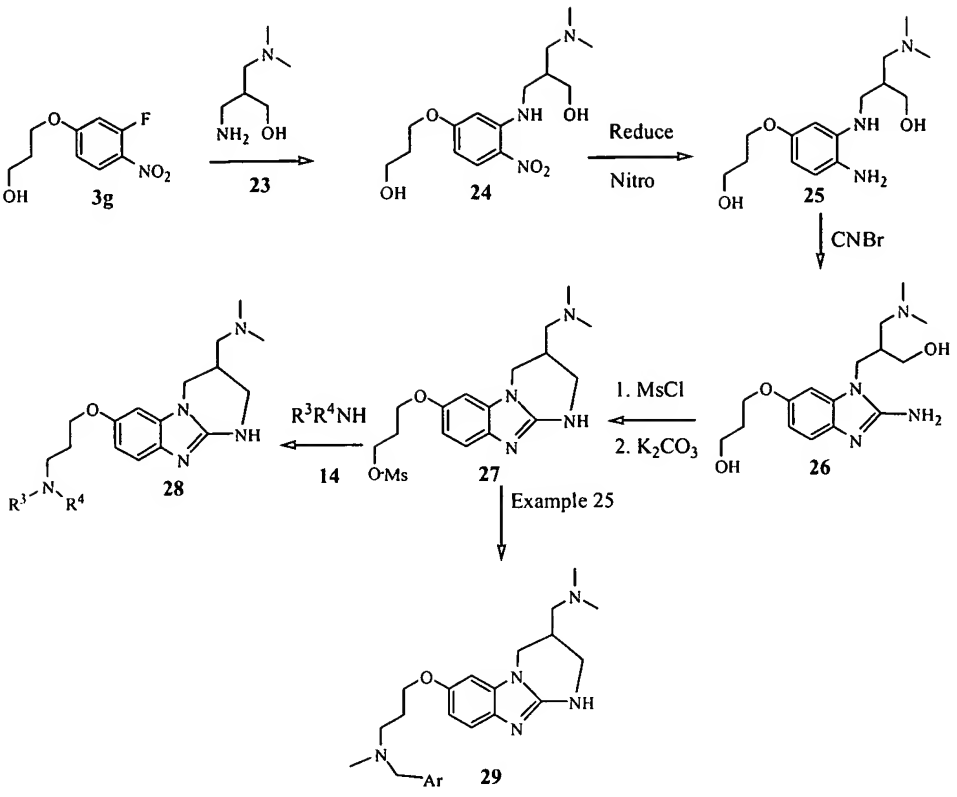
[0074] 2-aminobenzimidazole **12r** was prepared as per the procedure described in Example 2, except that the acid-base extraction after the Mitsunobu alkylation (step 1) was replaced by column chromatography. Also, the cyanogens bromide cyclization was carried out in the presence of K_2CO_3 (2 eq.). Purification by chromatography on silica gel (15% MeOH/1% Et_3N/THF) provided **12r**.

[0075] Purified **12r** obtained above was dissolved in a mixture of CH_2Cl_2 (5 mL) and TFA (5 mL) and the reaction was stirred at room temperature for 12h. The reaction was concentrated under vacuum and the residue was dissolved in CH_2Cl_2 and the organic phase was washed with 4M NaOH and then with brine, after which it was dried over $MgSO_4$ and concentrated to provide crude **19**.

[0076] A solution of crude **19** and DIPEA (0.25 mmol, 0.032 mL) in DMF (0.3 mL) was cooled in an ice bath and 1*H*-pyrazole-1-carboxamidine hydrochloride (0.25 mmol, 0.037g) was added as a solid to the reaction. After stirring for 12 h at room temperature, the reaction was diluted with CH_2Cl_2 and washed with 4M NaOH and then with brine, after which it was dried over $MgSO_4$ and concentrated to provide guanidine **20**, which was purified by preparative rpHPLC.

[0077] **N-{3-[2-dimethylamino-propyl)-3H-benzoimidazol-5-yloxy]-propyl}-guanidine (20a')** was prepared according to the procedure described in Example 5 and purified by preparative rpHPLC to provide 20.5 mg of **20a'·3TFA**. LCMS: LC retention time 0.63 min.; MS (ES^+) 334.1 (MH^+). ^1H NMR (200 MHz, CD_3OD) d7.3 (d, 1H), 7.2 (d, 1H), 6.95 (dd, 1H), 4.2 (m, 4H), 3.4 (t, 2H), 3.2 (m, partially overlapped 2H), 2.95 (s, 6H), 2.24 (m, 2H), 2.15 (m, 2H).

[0078] **Example 6 – Synthesis of N1-N2 cyclic 2-aminobenzimidazoles**



[0079]

[0080] 2-hydroxymethyl-acrylonitrile (37 mmol, 3.1 g, prepared as per procedure described by Csuk et al. in *Tetrahedron*, **1996**, *52*, 9759-9776) was treated with dimethylamine (25 mL of a 2M solution in THF) and the reaction was heated in a sealed vessel at 45°C for 14h. The reaction was cooled to room temperature and all the volatiles were removed by concentration under vacuum. The crude 2-hydroxymethyl-3-dimethylaminopropionitrile thus obtained was dissolved in dry THF (50-60 mL) and this solution was added dropwise to a cold (-78°C) suspension of LAH (6g) in THF (300 ML). The reaction was gradually warmed to room temperature and stirred for an additional 12 h at room temperature. The reaction was then cooled in an ice bath and very carefully quenched by the sequential addition of H_2O (6 mL), 4M NaOH (6 mL) and H_2O (18 mL). The white slurry thus obtained was filtered through celite and the filter bed was washed with additional CH_2Cl_2. The entire filtrate was then concentrated under vacuum to provide crude amine **23**.

[0081] A mixture of crude **23** was obtained above, nitrophenol **3g** (40 mmol, 8.6 g) and K_2CO_3 (60 mmol, 8.3 g) in toluene (80 mL) was refluxed for 3 h. The reaction was then poured into water and extracted with EtOAc. The crude material was purified by flash chromatography (10% MeOH / 1% NH_4OH / CH_2Cl_2 to provide **24**. LCMS: LC retention time 2.16 min.; MS (ES^+) 328.1 (MH^+).

[0082] 10% Pd/C (200 mg) and crude **24** obtained above was dissolved in EtOH (50 mL) and the mixture was hydrogenated at atmospheric pressure for 12 h at room temperature. The reaction was filtered through celite and concentrated to provide **25** as a dark oil.

[0083] Crude **25** obtained above was dissolved I n EtOH (30 mL) and treat with CNBr (22.5 mmol, 2.36 g). The reaction was stirred for 12 h after which it was diluted with 4M NaOH until strongly basic (pH > 12) and extracted with CH_2Cl_2 (3X). The combined organic layers were washed with brine and dried over $MgSO_4$, then concentrated to provide **26** as a dark oil. The crude residue was purified by flash column

chromatography on neutral alumina (5-10% MeOH / 1% NH_4OH / CH_2Cl_2) to provide **26** (0.94 g) as a dark oil. LCMS: LC retention time 0.507 min.; MS (ES^+) 323.1 (MH^+).

[0084] A solution of **26** (2.9 mmol, 0.94 g), DMAP (5 mg) and triethylamine (7.5 mmol, 1.05 mL) in dry CH_2Cl_2 (5 mL) was cooled in an ice bath and methanesulfonyl chloride (5.98 mmol, 0.46 mL) was added dropwise over 5 min. The reaction was stirred for 2h, after which it was diluted with CH_2Cl_2 and the organic layer was washed with saturated Na_2CO_3, then brine, then dried over $MgSO_4$ and concentrated to provide crude **27**. The crude material obtained above was dissolved in DMF (11 mL) and this solution was distributed to 11 vials (1 ML each). K_2CO_3 (0.5 mmol, 0.07 g) and the desired primary or secondary amine (0.4 mmol) was added to each reaction vessel. The individual reactions were heated at 60°C for 14 h, after which the contents of each vessel was diluted with CH_2Cl_2 and the organic layer was washed with 4M NaOH, then with brine, and then dried over $MgSO_4$ and concentrated to provide crude **28**, which was further purified by preparative reverse phase HPLC.

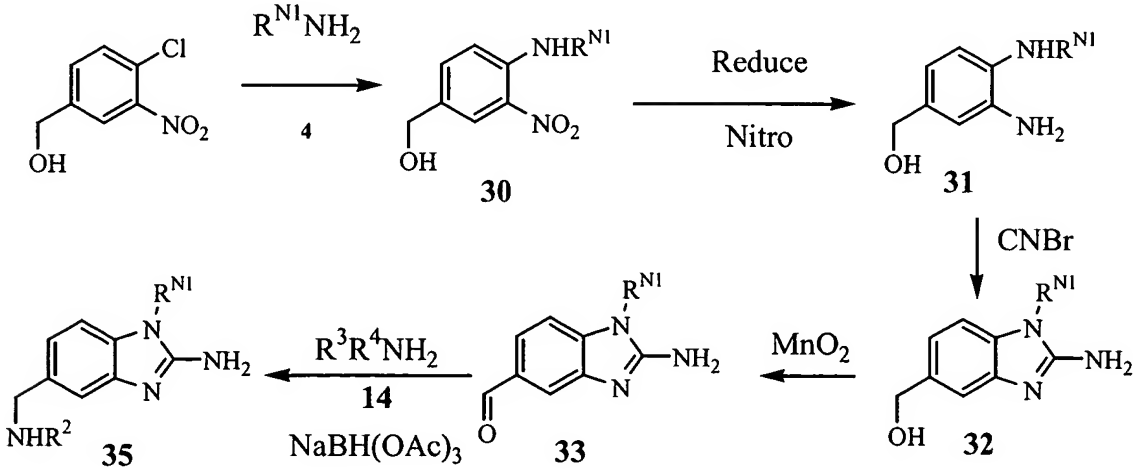
[0085] **Dimethyl-{7-[3-(4-pyrrolidin-1-yl-piperidin-1-yl)-propoxy]-1,2,3,4-tetrahydrobenzo[4,5]-imidazo[1,2-a]pyrimidin-3-ylmethyl}-amine (28ai)** was prepared according to the procedure described in Example 6 and purified by preparative rpHPLC to provide 24 mg of **28ai·3TFA**. LCMS: LC retention time 0.59 min.; MS (ES^+) 441.2 (MH^+).

[0086] Heteroaryl or aryl groups (**29**) could be appended onto the alkyloxy chain at C6 by processing compound **27** per the procedure described in Example 4.

[0087] **[3-(3-Dimethylaminomethl-1,2,3,4-tetrahydro-benzo[4,5]imidazo[1,2-a]pyrimidin-7-yloxy)-propyl]-methyl-(1H-pyrrol-2-ylmethyl)-amine (29i)** was prepared according to the procedure described in Example 6 and purified by preparative rpHPLC to provide 25 mg of **29i·3TFA**. LCMS: LC retention time 1.32 min.; MS (ES^+) 397.2 (MH^+).

[0088] **Example 7 – C_5 aminomethl-2-aminobenzimidazoles – Reductive amination.**

[0089] **Example 7**

[0090] 

[0091] A mixture of 3-hydroxymethyl-6-chloronitrobenzene (10.6 mmol, 2 g) and amine **4** (12 mmol) in toluene (10 mL) was refluxed for 2 h. The reaction was then diluted with EtOAc and the organic phase was washed with water and then with brine, then dried over $MgSO_4$ and concentrated to provide **30**.

[0092] Crude **30** obtained above was reduced and cyclized with CNBr according to the procedure described in Example 1 to provide **32**.

[0093] A mixture of **32** (2.5 mmol, 0.63 g) and MnO_2 (10 mmol, 0.87g) in CH_2Cl_2 was refluxed for 6 h. The reaction was filtered through celite and concentrated to provide **33**, which was purified by chromatography on neutral alumina (5% MeOH / CH_2Cl_2).

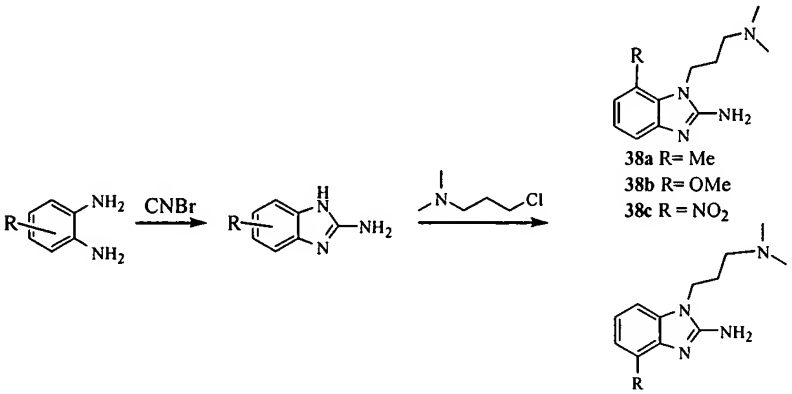
[0094] A mixture of aldehyde **33** (0.3 mmol, 0.075 g), 3-dimethylaminopropylamine (0.9 mmol, 0.15 mL), glacial acetic acid (1 drop) and $NaBH(OAc)_3$ (0.45 mol, 0.1 g) in CH_2Cl_2 (2 mL) was stirred overnight. The reaction was diluted with CH_2Cl_2 and the organic phase was washed with water, then with brine, then dried over $MgSO_4$ and then concentrated to provide **35**, which was purified by rpHPLC.

[0095] **1-(3-Dimethylamino-propyl)-5-morpholin-4-ylmethyl-1H-benzoimidazol-2-ylamine (35ra')** was prepared according to the procedure described in Example 7 and purified by preparative rpHPLC to provide 29 mg of **35ra'·3TFA**. LCMS: LC retention time 0.52 min.; MS (ES^+) 318.2 (MH^+).

[0096] **1-(3-Dimethylamino-propyl)-5-(3-dimethylaminopropyl)-aminomethyl-1H-benzoimidazol-2-ylamine (35qa')** was prepared according to the procedure described in Example 7 and purified by preparative rpHPLC to provide 32 mg of **35aqa'·3TFA**. LCMS: LC retention time 0.41 min.; MS (ES^+) 333.2 (MH^+).

[0097] **Example 8 Alkylation of 2-aminobenzimidazole**

[0098] **Example 8**



[0099]

[00100] A mixture of 2,3-dinitrophenol (5.43 mmol, 1 g), iodomethane (21.7 mmol, 1.35 mL) and K_2CO_3 (21.7 mmol, 2.99 g) was stirred at room temperature for 14 h. The reaction was then filtered through celite and the filter bed was washed with additional acetone. The filtrate was concentrated to provide 2,3-dinitroanisole (100%), which was dissolved in a mixture of EtOH / H_2O (1:1, 20 mL) and Fe (19.4 mmol,

1.06 g) and concentrated HCl (8 drops) was added. The mixture was refluxed for 90 min., after which it was filtered through celite and the filter bed was washed with additional EtOH. The filtrate was concentrated and basified (pH > 12) with 4 M NaOH and the aqueous layer was extracted with CH_2Cl_2. The organic phase was then separated and washed with brine, dried $MgSO_4$ and concentrated to provide 2,3-diaminoanisole **36b** (0.65 g).

[00101] Cyanogen bromide (7.05 mmol, 0.74 g) was added to a solution of 2,3-diaminoanisole (4.7 mmol, 0.65 g) in EtOH (10 mL) and the reaction was stirred at room temperature for 14 h. The reaction was diluted with H_2O and then basified (pH > 12) using 4 M NaOH. The aqueous layer was extracted with CH_2Cl_2. The organic phase was then separated and washed with brine and then dried over $MgSO_4$ and then concentrated to provide 2-aminobenzimidazole **37b**.

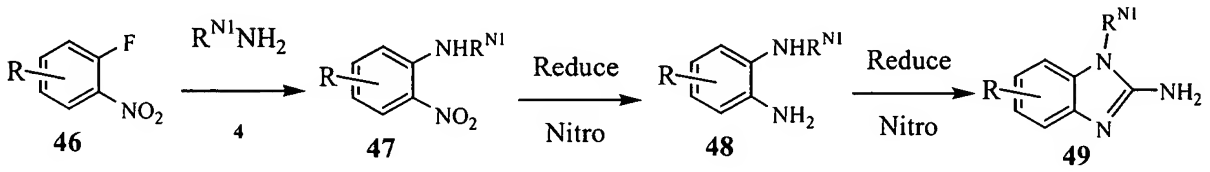
[00102] A mixture of crude **37** obtained above, KOH (4 mmol, 0.224 g) and dimethylaminopropyl chloride. HCl (2 mmol, 0.32 g) was refluxed in EtOH (5 mL) for 14 h. The reaction was diluted with CH_2Cl_2 and the organic phase was washed with water and then with brine and then dried over $MgSO_4$ and then concentrated to provide a mixture of **38b** and **39b**, which was separated by preparative rpHPLC.

[00103] **1-(3-Dimethylamino-propyl)-4-methoxy-1H-benzoimidazol-2-ylamine (38b) and 1-(3-Dimethylamino-propyl)-4-methoxy-1H-benzoimidazol-2-ylamine (39b)** were purified by preparative rpHPLC to provide **38b·2TFA** (8 mg) and **39b·3TFA** (29.5 mg). **38b** LCMS: LC retention time 1.36 min.; MS (ES^+) 249.1 (MH^+). **39b** LCMS: LC retention time 1.46 min.; MS (ES^+) 249.1 (MH^+).

[00104] The nitro-substituted 2-aminobenzimidazoles **38c** and **39c** could be reduced to the corresponding diaminobenzimidazoles using 10% Pd/C and hydrogen gas.

[00105] **Example 9 – Synthesis of 2-aminobenzimidazoles**

[00106] **Example 9**



[00107] R = Cl, Br, CN, CO_2Me, CF_3, Me, NO_2, etc.

[00108] Amine **4** (2.4 mmol was added to amixture of fluoronitro compound **46** (2 mmol) and $CaCO_3$ (0.4 g, 4 mmol) in CH_2Cl_2 (2 mL) at room temperature. The reaction was stirred for 12 h, after which it was filtered through celite and the filter pad was washed with additional CH_2Cl_2. The solvent was removed by concentration and the crude product was hydrogenated using 10% Pd/C (50 mg) and H_2 gas (Balloon) in EtOH (10 mL) for 12 h at room temperature, after which the reacton was filtered through celite and concentrated. The crude product thus obtained was suspended in water (2 mL) and treated with CNBr (4 mmol, 0.41 g) and the reaction was stirred for 12 h at room temperature. The reaction was basified using

4M NaOH (pH > 12) and the aqueous layer was extracted with CH_2Cl_2. The layers were separated and the organic layer was dried $MgSO_4$ and concentrated. The material thus obtained was washed with ether (2-4 mL) and dried under high vacuum to provide the 2-aminobenzimidazole **49**.

[00109] When the R group is nitro, the 2-aminobenzimidazole **49** can be reduced to the corresponding diamino-benzimidazole using 10% Pd/C (catalytic amount) and hydrogen gas at atmospheric pressure. Filtering the reaction through celite followed by solvent removal under vacuum provides the final diaminobenzimidazole in essentially quantitative yield.

[00110] **1-(3-Dimethylaminopropyl)-5-trifluoromethyl-1H-benzoimidazol-2-ylamine (49d)** was prepared by procedure described in Example 9. ^{1}H NMR (200 MHz, $CDCl_3$) d 7.62 (1H, s), 7.29 (1H, d, J = 8.3), 7.09 (1H, d, J=8.4), 6.44 (2H, s, br), 4.05 (2H, m), 2.26 (3H, s), 2.22 (2H, m), 1.97 (2H, m), 1.97 (2H, m). LCMS: LC retention time 1.57 min.; MS (ES^+) 287.1 (MH^+).

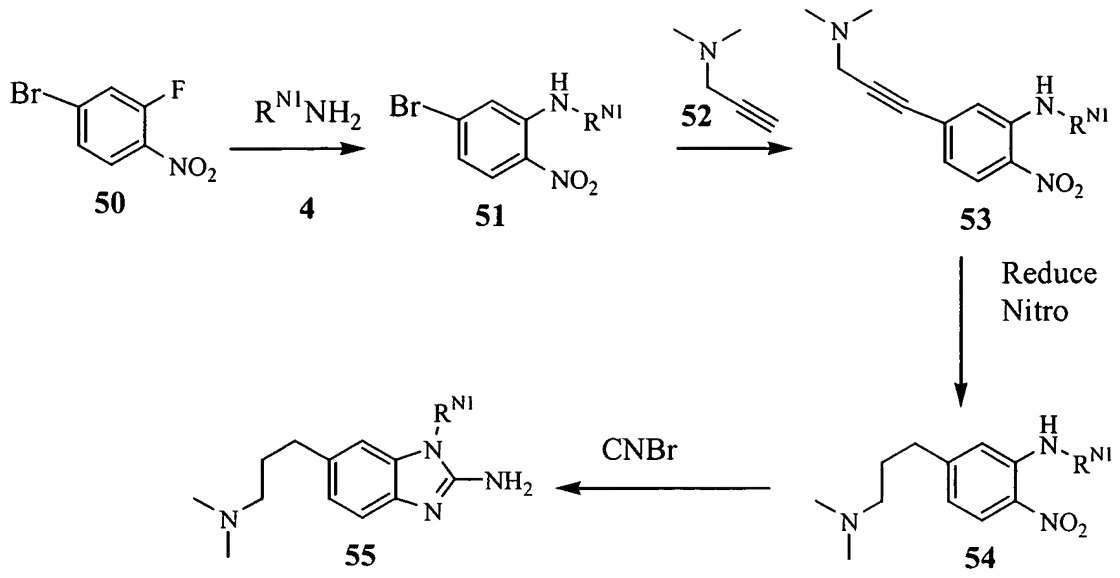
[00111] 10% Pd/C (200 mg) and crude **24** obtained above was dissolved in EtOH (50 mL) and the mixture was hydrogenated at atmospheric pressure for 12 h at room temperature. The reaction was filtered through celite and concentrated to provide **25** as a dark oil.

[00112] Dimethyl-{7-[3-(4-pyrrolidin-1-ylpiperidin-1-yl)propoxy]-1,2,3,4-tetrahydro-benzo[4,5]imidazo[1,2-a]pyrimidine-3-ylmethyl}-amine (28ai) was prepared according to the procedure described in Example 10 and purified by preparative reverse phase HPLC to provide 25 mg of 29i·3TFA. LCMS: LC retention time 1.32 min.; MS (ES^+) 397.2 (MH^+).

[00113] When the R group is nitro, the 2-aminobenzimidazole **49** can be reduced to the corresponding diaminobenzimidazole using 10% Pd/C (catalytic amount) and hydrogen gas at atmospheric pressure. Filtering the reaction through celite followed by solvent removal under vacuum provides the final diaminobenzimidazole in essentially quantitative yield.

[00114] **(1-(3-Dimethylamino-propyl)-5-trifluoromethyl-1H-benzoimidazol-2-ylamine (49d)** was prepared by procedure described in Example 9. ^{1}H NMR (200 MHz, $CDCl_3$) d 7.62 (1H, s), 7.29 (1H, J = 8.3), 7.09 (1H, J = 8.4), 6.44 (2H, s, br), 4.05 (2H, m), 2.26 (3H, s), 2.22 (2H, m), 1.97 (2H, m), 1.97 (2H, m). LCMS: LC retention time 1.57 min.; MS (ES^+) 287.1 (MH^+).

[00115] **Example 10 – Synthesis of 6-alkylaminoalkyl-2-aminobenzimidazoles**

[00116] 

[00117] Amine **4** (16.7 mmol) was added to mixture of 4-bromo-2-fluoronitrobenzene (11.18 mmol, 2.45 g) and $CaCO_3$ (0.4 g, 4 mmol) in CH_2Cl_2 (2 mL) at rt. The reaction was stirred for 12 h, after which it was filtered through celite and the filter pad washed with additional CH_2Cl_2. The filtrate was then washed with water, then brine, then dried over $MgSO_4$ and concentrated to provide **51**.

[00118] A mixture of **51** (3.67 mmol, 1 g), copper iodide (0.183 mmol, 0.034 g), $Pd(Ph_3)_3$ (0.09 mmol, 0.103 g) and triethylamine (14.68 mmol, 2 mL) in dry THF (36 mL) was cooled in an ice bath. Alkyne **52** (5.5 mmol, 0.63 mL) was added to the reaction and the mixture was stirred at room temperature for 14 h. The solvent was the removed under vacuum and the residue was dissolved in CH_2Cl_2 and the organic phase was washed with water, then brine, and then dried and concentrated to provide **53**.

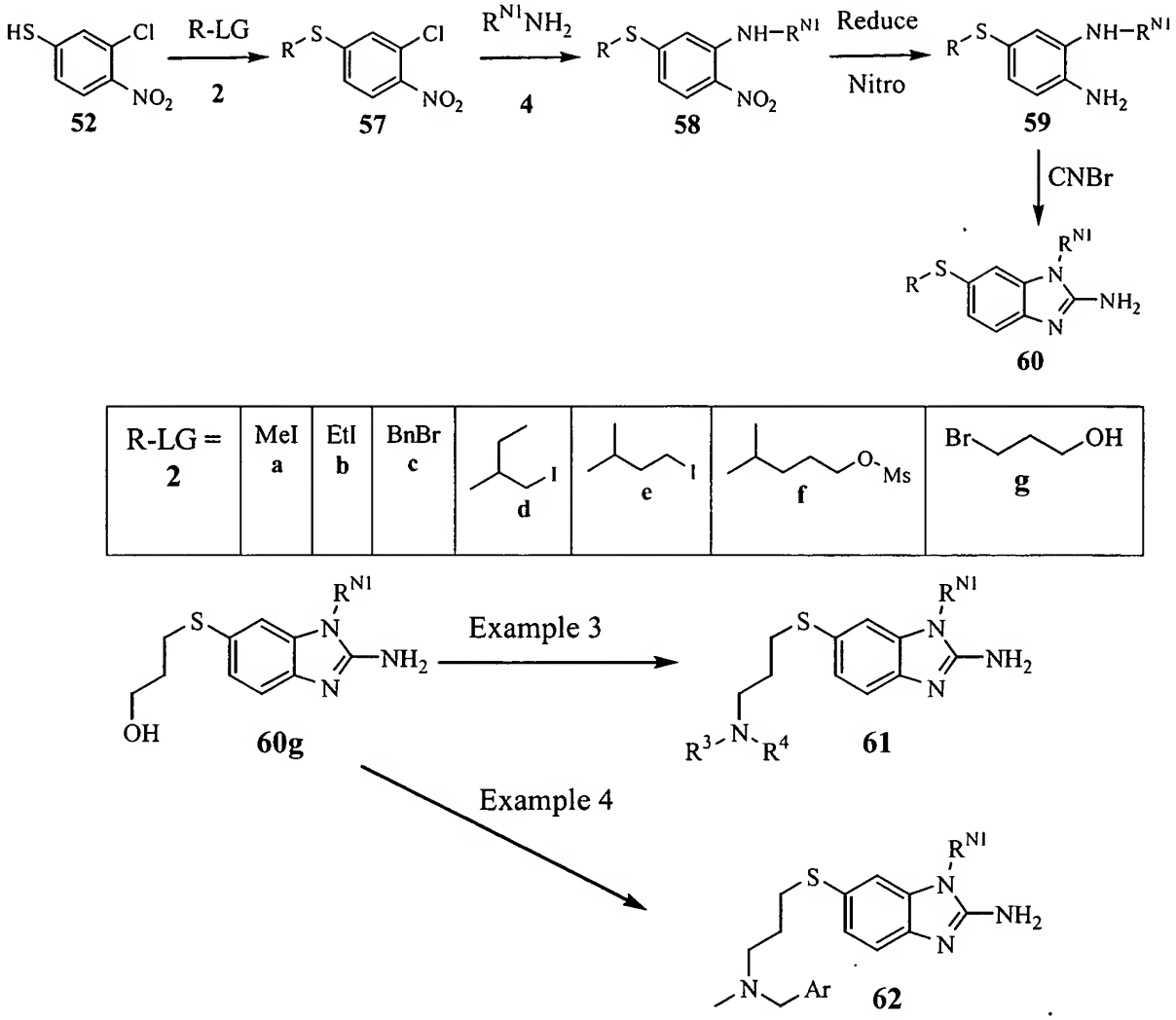
[00119] 10 % Pd/C (100 mg) and crude **53** obtained above was dissolved in EtOH (20 mL) and the mixture was hydrogenated at atmospheric pressure for 12 h at room temperature. The reaction was filtered through celite and concentrated to provide **54** as a dark oil.

[00120] Crude **6** obtained above was dissolved in EtOH (8 mL) and treated with CNBr (4.9 mmol, 0.52 g). The reaction was stirred for 12 h, after which it was diluted with 4 M NaOH until strongly basic (pH > 12) and extracted with CH_2Cl_2 (3X). The combined organic layers were washed with brine, dried $MgSO_4$ and concentrated to provide **55** as a dark oil, which was purified by preparative rpHPLC.

[00121] **1,6-Bis-(3-dimethylamino-propyl)-1H-benzoimidazole-2-ylamine (55a')** was prepared according to the procedure described in Example 10 and purified by preparative reverse phase HPLC to provide mg of **55a'3TFA**. LCMS: LC retention time 0.57 min.; MS (ES^+) 352.1 (MH^+).

[00122] Example 11 – Synthesis of 6-alkylaminoalkylthio-2-aminobenzimidizole

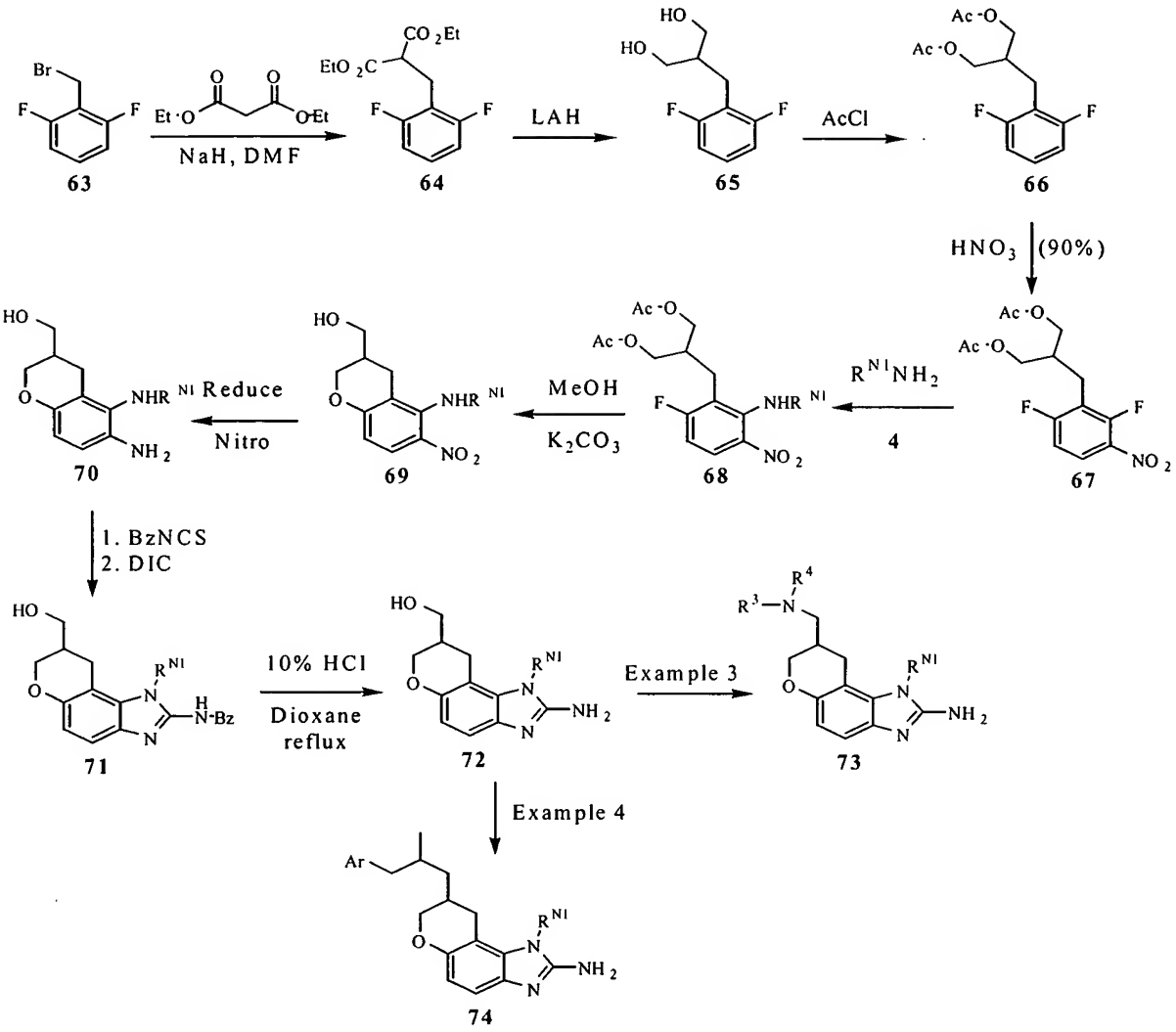
Example 11



[00123] 2-amionbenzimidazole **60** is prepared starting from 3-chloro-4-nitrothiophenol according to the procedure described in Example 1. 2-aminobenzimidazoles **61** and **62** are prepared starting from **60g** according to the procedure described in Example 3 and Example 4 respectively.

[00124] **Example 12 – Synthesis of C6 – C7 constrained 2-amionbenzimidazoles**

[00125] **Example 12**



[00126]

[00127] Diethylmalonate (40 mmol, 6.1 mL) was added to a suspension of NaH (44 mmol, 1.76 g of a 60% dispersion in mineral oil) in dry THF (60 mL). After stirring for 10 min. at room temperature, the suspension turned to a clear solution (with evolution of hydrogen), which was then cooled to -78°C. Bromide **63** (40 mmol, 8.26 g) in dry THF (20 mL) was then added dropwise over 20 minutes to the reaction. The ice bath was removed and the reaction was gradually allowed to warm up to room temperature over 2-3 h. The reaction was then diluted with CH_2Cl_2, extracted with water (2X) and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated to provide **64**, wich was used without any further purification.

[00128] Crude **64** obtained above in dry THF (60 mL) was added dropwise to a cold (-78°C) suspension of LAH (4.48 g) in THF (300 mL). The reaction was gradually allowed to warm to room temperature over 14 h, after which it was recooled in an ice bath. Water (4.5 mL) was carefully added to the reaction, followed by sequential addition of 4M NaOH (4.5 ml) and water (13.5 mL). The reaction was further diluted with ether (100 mL), filtered through celite and the filter bed was thoroughly washed with additional ether. The filtrate was concentrated and the residue was purified by column chromatography to provide **65** (50% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) d 7.16 (m, 1H), 6.85 (m, 2H), 3.8 (m, 4H), 2.7 (d, 2H), 2.0 (m, 1H).

[00129] A solution of acetyl chloride (59 mmol, 4.21 mL) in dry CH_2Cl_2 (60 mL) was added dropwise to a cold (0°C) solution of diol **65** (25.6 mmol, 5.18 g), triethylamine (64 mmol, 8.9 mL) and DMAP (2.56 mmol, 0.32 g) in dry CH_2Cl_2 (190 mL). The reaction was then stirred for an additional 14 h at room temperature, after which it was diluted with CH_2Cl_2 and the organic phase was washed with 5% HCl, then with brine, then dried over $MgSO_4$ and concentrated to provide **66**, which was used without any further purification. ^{1}H NMR (300 NMR, $CDCl_3$) d 7.16 (m, 1H), 6.85 (m, 2H), 4.0 (d, 4H), 2.78 (d, 2H), 2.37 (m, 1H), 2.02 (s, 6H).

[00130] Crude **66** (25.8 mmol, 7.38 g) obtained above was cooled in an ice bath and fuming nitric acid (15 mL) was added over 5 min. The reaction was stirred for 1 h in the ice bath, after which it was diluted with ice water. The cold aqueous solution was then extracted with CH_2Cl_2 (3X) and the combined organic phases were washed with brine, then dried over $MgSO_4$ and concentrated to provide **67**, which was used without any further purification. ^{1}H NMR (300 NMR, $CDCl_3$) d: 8.0 (m, 1H), 7.0 (m, 2H), 4.06 (d, 4H), 2.85 (d, 2H), 2.4 (m, 1H), 2.02 (s, 6H).

[00131] Amine **4** (30 mmol) was added to a suspension of crude **67** (20 mmol) and $CaCO_3$ (28 mmol, 2.8 g) in CH_2Cl_2 (40 mL). The reaction was stirred for 14 h at room temperature, after which it was diluted with CH_2Cl_2 and the organic phase was washed with water, then with brine, then dried over $MgSO_4$ and concentrated to provide nitroaniline **68**, which was used without any further purification.

[00132] A suspension of crude nitroaniline **68** (18.4 mmol) obtained above and K_2CO_3 (54 mmol, 7.46 g) in MeOH (180 mL) was stirred at room temperature for 72 h and 40°C for 24 h. The solvent was then removed by concentration under reduced pressure and the residue was dissolved in water an the aqueous solution was extracted with CH_2Cl_2 (3X). The combined organic layers were then washed with brine, then dired over $MgSO_4$ and concentrated to provide crude **69**, which was used without any further purification.

[00133] A solution of crude **69** in EtOH (200 mL) was treated with 1% Pd/C (0.46 g) and the mixture was hydrogenated at atmospheric pressure for 20 h to provide crude **70**, which was used without any further purification.

[00134] Benzoylisothiocyanate (9.76 mmol, 1.31 mL) was added to a cold (0°C) solution of crude **70** in CH_2Cl_2 (20 mL) and the mixture was stirred for an additional 2 h at room temperature. Diisopropylethylamine (29.28 mmol, 5.1 mL) was then added to the reaction, followed by diisopropylcarbodimide (14.64 mmol, 2.3 mL) and the whole was then stirred at room temperature for 14 h. The reaction was diluted with CH_2Cl_2 and the organic phase was washed with water. The CH_2Cl_2 layer was then extracted with 5% HCl (2X) and the acidic aqueous layers were combined and washed with EtOAc (1X). The acidic aqueous layer was then basified with solid NaOH until strongly basic (pH > 12) and extracted with CH_2Cl_2 (3X). The combined organics were further washed with brine, dried over $MgSO_4$ and concentrated to provide **71**, which was used without any further purification.

[00135] Crude **71** (1.62 g, 4 mmol) was dissolved in a mixture of 1,4-dioxane (2.4 mL) and 10% HCL (5.6 mL) and the reaction was refluxed for 14 h. The reaction was then cooled and basified with solid NaOH

until strongly basic (pH > 12) and the aqueous layer was extracted with CH_2Cl_2 (3X). The combined organic layers were then washed with brine, dried over $MgSO_4$ and concentrated to provide **72.**

[00136] **8-Dimethylaminomethyl-1-(3-dimethylamino-propyl)-1,7,8,9-tetrahydro-chromeno[5,6-d]imidazol-2-ylamine (73a')** was prepared from **72a'** according to the procedure described in Example 3, using dimethylamine (excess) as the nucleophile for mesylate displacement and purified by preparative rpHPLC to provide **73a'·CH_3CO_2H** (44.4 mg). LCMS: LC retention time 0.34 min.; MS (ES^+) 332.2 (MH^+).

[00137] **1-(3-Dimethylaminopropyl)-8-{[methyl-(1-methyl-1H-pyrrol-2-ylmethl)-amino]-methyl}-1,7,8,9-tetrahydro-chromeneo[5,6-d]imidazol-2-ylamine (74a')** was prepared from **72a'** according to the procedure described in Example 4 and purified by preparative rpHPLC to provide **74a'· CH_3CO_2H** (21.1 mg). LCMS: LC retention time 0.51 min.; MS (ES^+) 411.2 (MH^+).

[00138] **Example 13 – Synthesis of extended tether C6-C7 constrained 2-aminobenzimidazoles.**

[00139]

[00140] Methanesulfonyl chloride (1.79 mmol, 0.14 mL) was added dropwise to a cold solution (0°C) of 2-aminobenzimidazole **72** (1.19 mmol, 0.36 g, prepared according to Example 12), triethylamine (1.79 mmol, 0.25 mL) and dimethylaminopyridine (10 mg) in dry CH_2Cl_2 (2 mL). The reaction was stirred for 2 h, after which it was diluted with CH_2Cl_2 and the organic phase was extracted with water, then with brine, then dried over $MgSO_4$ and concentrated to provide crude mesylate mixture **75** and **76.**

[00141] The crude material obtained above was dissolved in DMF (1 mL) and KCN (68 mg) was added and the reaction was heated for 14 h at 60°C. The reaction was then cooled and diluted with water and extracted with CH_2Cl_2 to provide the corresponding cyanide **77.**

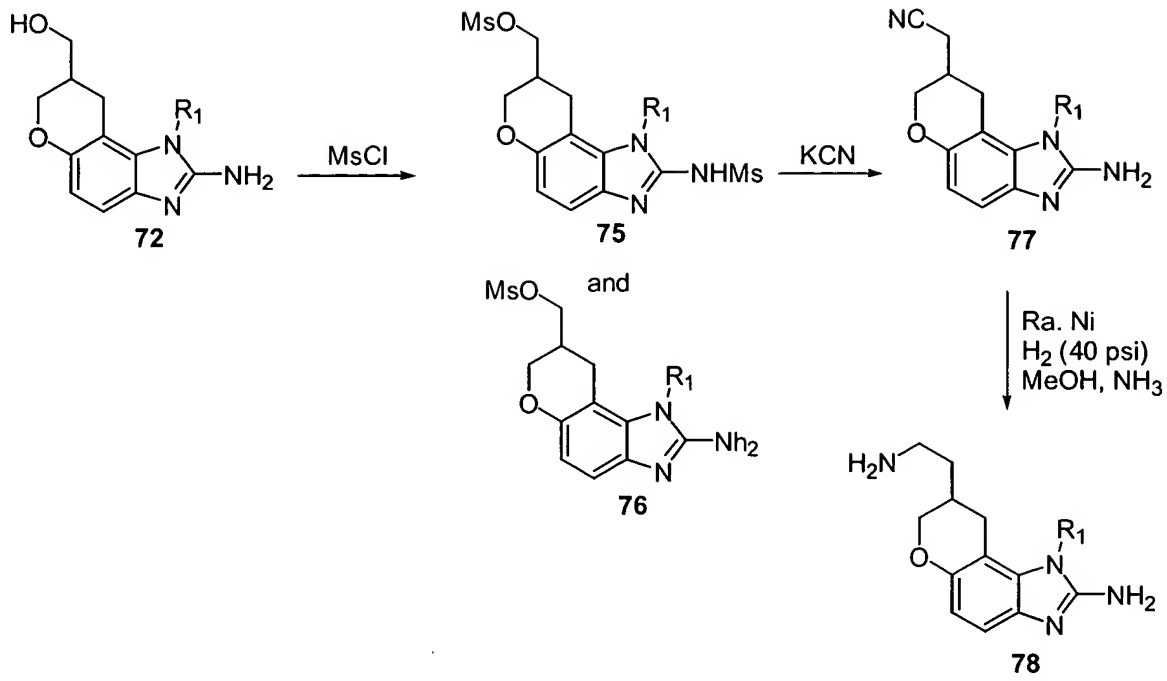
[00142] The crude **77** obtained above was dissolved in a mixture of MeOH (0.8 mL) and strong NH_3 solution (0.2 mL) and treated with Raney Nickel catalyst. The whole mixture was then hydrogenated to provide amine **78,** which was isolated by filtration through celite.

[00143] **8-(2-Aminoethl)-1-(3-dimethylaminopropyl)-1,7,8,9-tetrahydrochromeno[5,6-d]imidazole-2-ylamine (78a')** was prepared using the procedure described in Example 34 and purified by preparative rpHPLC to provide **78a' ·CH_3CO_2H** (12.6 mg). LCMS: LC retention time 0.41 min.; MS (ES^+) 318.2 (MH^+).

[00144] In the study design, 3-4 female mice/group were dosed with 0, 5 or 45 mg/kg of IBIS00553642, IBIS000408094, or IBIS00405746 for 3 days (i.p.). Clinical signs, body weights, clinical pathology, organ weights, and histopathology endpoints were evaluated.

[00145] **Example 13 – Synthesis of extended tether C6 - C7 constrained 2-aminobenzimidazoles.**

[00146]



[00147]

[00148]

[00149] Methanesulfonyl chloride (1.79 mmol, 0.14 mL) was added dropwise to a cold solution (0 °C) of 2-aminobenzimidazole 72 (1.19 mmol, 0.36 g, prepared according to example 12), triethylamine (1.79 mmol, 0.25 mL) and dimethylaminopyridine (10 mg) in dry CH_2Cl_2 (2 mL). The reaction was stirred for 2 hours after which it was diluted with CH_2Cl_2 and the organic phase was extracted with water, brine, dried (MgSO4) and concentrated to provide crude mesylate mixture 75 and 76.

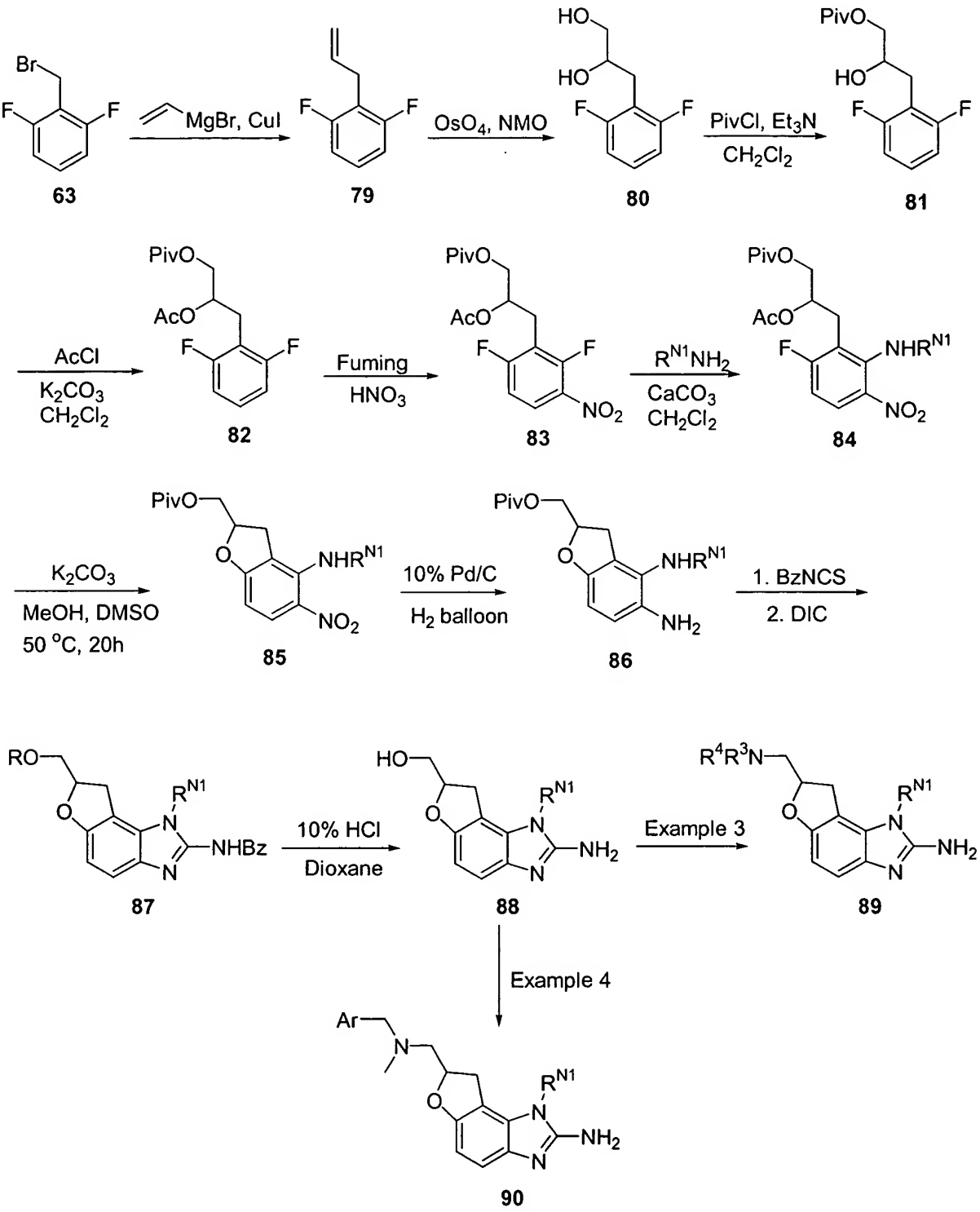
[00150] The crude material obtained above was dissolved in DMF (1 mL) and KCN (68 mg) was added and the reaction was heated for 14h at 60°C. The reaction was then cooled and diluted with water and extracted with CH_2Cl_2 to provide the corresponding cyanide 77.

[00151] The crude cyanide **77** obtained above, was dissolved in a mixture of MeOH (0.8 mL) and strong NH_3 solution (0.2 mL) and treated with Raney Nickel catalyst. The whole mixture was then hydrogenated to provide amine **78**, which was isolated by filtration through celite.

[00152] **8-(2-Amino-ethyl)-1-(3-dimethylamino-propyl)-1,7,8,9-tetrahydro-chromeno[5,6-d]imidazol-2-ylamine (78a')** was prepared using the procedure described in example 13 and purified by reverse phase preparative HPLC to provide **78a'·3CH_3CO_2H** (12.6 mg). LCMS: LC retention time 0.41 min.; MS (ES^+) 318.2 (MH^+).

[00153] **Example 14 – Synthesis of C6 - C7 constrained 2-aminobenzimidazoles (benzofuran series)**

[00154]



[00155]

[00156]

[00157] Vinyl Magnesium Bromide (22.5 mL of a 1M solution in THF, 22.5 mmol) was slowly added to a cold (0 °C), stirred suspension of CuI (1.5 mmol, 0.285 g) and 2,2'-dipyridyl (1.5 mmol, 0.234 g) in dry THF (40 mL). After stirring for 30 min, the reaction was cooled to –78 °C and bromide **63** was added dropwise as a solution in dry THF (10 mL). The reaction mixture was allowed to warm gradually over 3 h after which it was quenched with sat. NH_4Cl solution. The reaction was then diluted with EtOAc and the

aqueous phase was separated. The organic phase was then washed with additional sat. NH_4Cl, brine, dried ($MgSO_4$) and concentrated to provide crude **79**, which was used without any further purification.

[00158] Crude **79** obtained above was dissolved in acetone (50 mL) and the reaction mixture was treated with OsO_4 (catalytic) and N-methylmorpholine-N-oxide (1.5g). After stirring for 14 h at rt, the reaction was evaporated to dryness and purified by column chromatography (silica gel, eluting with 10% EtOAc-50% EtOAc in hexanes) to provide diol **80** (1.35 g) as a white solid.

[00159] Trimethylacetyl chloride (5.2 mmol, 0.64 mL) was added dropwise to a cold (0 °C), stirred solution of diol **80** (3.47 mmol, 0.66 g), Et_3N (5.2 mmol, 0.73 mL) and catalytic DMAP in dry CH_2Cl_2 (5 mL). After stirring at rt for 14 h the reaction was diluted with CH_2Cl_2 and extracted with 5% HCl, sat. $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated to provide pivaloate ester **81**, which was used without any further purification.

[00160] Acetyl chloride (17 mmol, 1.15 mL) was added dropwise to a cold (0 °C), stirred suspension of pivaloate 81 (3.47 mmol) and K_2CO_3 (17.3 mmol, 2.39 g) in dry CH_2Cl_2 (5 mL). After stirring for 2h, the reaction was diluted with CH_2Cl_2, filtered (to remove K_2CO_3) and the organic layer was washed with water, dried ($MgSO_4$) and concentrated to provide crude **82**, which was used without any further purification.

[00161] Crude **82** (3.47 mmol) obtained above was cooled in an ice bath and fuming nitric acid (1 mL) was added over 5 min. After stirring at 0 °C for 1h, the reaction was diluted with water and extracted with CH_2Cl_2. The organic layer was separated and washed with brine, dried ($MgSO_4$) and concentrated to provide crude **83**, which was used without any further purification.

[00162] Amine **4** (4.9 mmol) was added to a suspension of crude **83** (3.27 mmol) and $CaCO_3$ (3.5 mmol, 0.35 g) in CH_2Cl_2 (6 mL). The reaction was stirred for 14 h at rt after which it was diluted with CH_2Cl_2 and the organic phase was washed with H_2O, brine, dried ($MgSO_4$) and concentrated to provide nitroaniline **84** which was used without any further purification.

[00163] A suspension of crude nitroaniline **84** (3.27 mmol) obtained above, K_2CO_3 (12.8 mmol, 1.74 g), MeOH (0.75 mL) in dry DMSO (30 mL) was stirred at rt for 2 h and 50 °C for 20h. The solvent was then removed by concentration under reduced pressure and the residue was dissolved in H_2O and the aq. solution was extracted with CH_2Cl_2 (3 X). The combined organic layers were then washed with brine, dried ($MgSO_4$) and concentrated to provide crude **85**, which was used without any further purification.

[00164] A solution of crude **85** in EtOH (50 mL) was treated with 10% Pd/C (0.1 g) and the mixture was hydrogenated at atmospheric pressure for 20 h to provide crude **86** which was used without any further purification.

[00165] Benzoylisothiocyanate (3.3 mmol, 0.44 mL) was added to a cold (0 °C) solution of crude **86** in CH_2Cl_2 (15 mL) and the mixture was stirred for an additional 2 h at rt. Diisopropylethylamine (13.2 mmol, 2.29 mL) was then added to the reaction followed by Diisopropylcarbodimide (4.6 mmol, 0.72 mL) and the reaction mixture was stirred at rt for 14h. The reaction was diluted with CH_2Cl_2 and the organic phase was

washed with H_2O. The CH_2Cl_2 layer was then extracted with 5% HCl (2X) and the acidic aqueous layers were combined and washed with EtOAc (1X). The acidic aqueous layer was then basified with solid NaOH till strongly basic (pH > 12) and extracted with CH_2Cl_2 (3X). The combined organics were further washed with brine, dried ($MgSO_4$) and concentrated to provide **87**, which was used without any further purification.

[00166] A mixture of crude **87** (3 mmol), 1,4-dioxane (2 mL) and 10% HCl (6 mL) was refluxed for 14h after which the reaction mixture was cooled and basified with solid NaOH till strongly basic (pH > 12). The aqueous layer was extracted with CH_2Cl_2 (3 X) and the combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated to provide **88**.

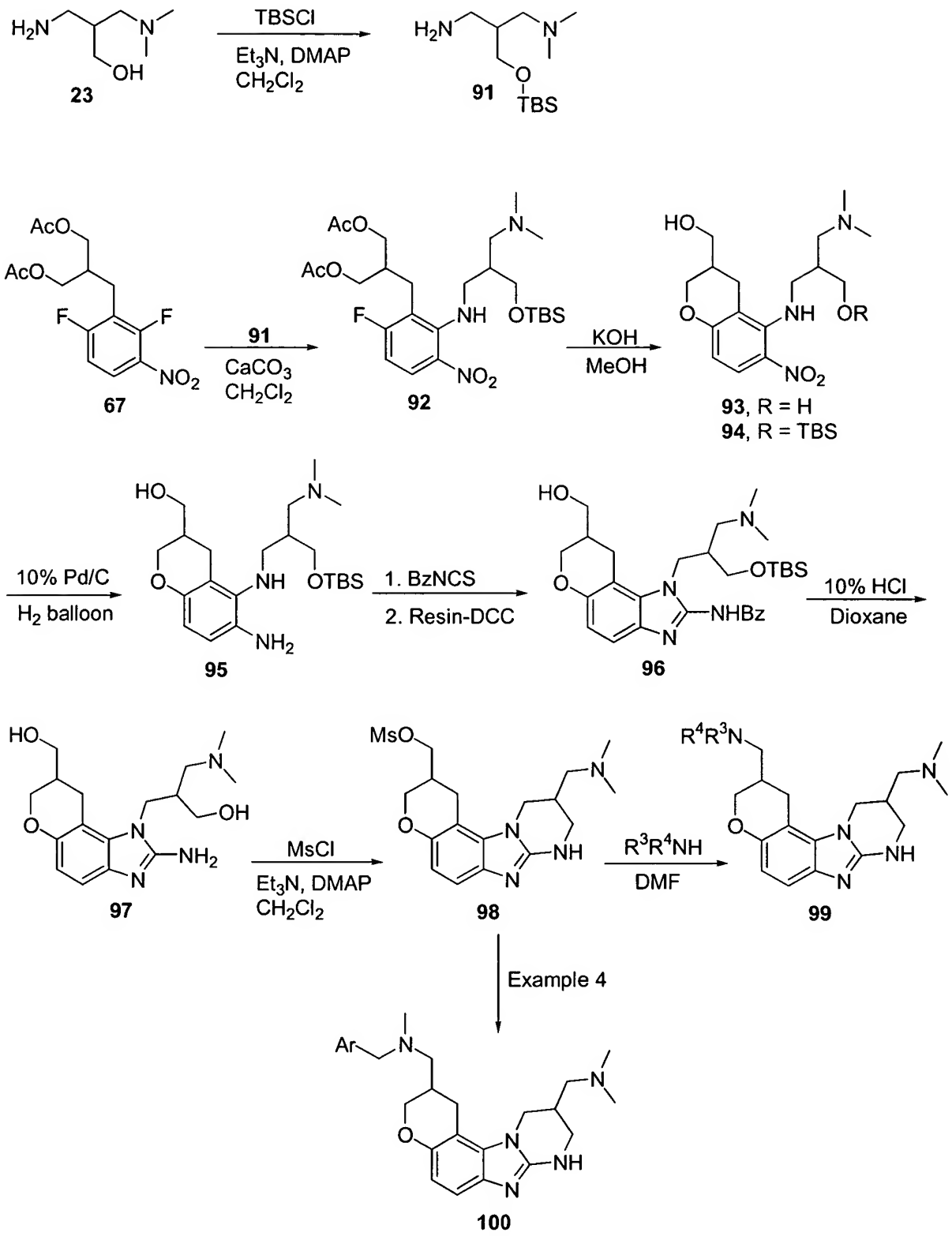
[00167] **[2-Amino-1-(3-dimethylamino-propyl)-7,8-dihydro-1H-6-oxa-1,3-diaza-as-indacen-7-yl]-methanol (88a')** was prepared according to the general procedure described in example 14 above. Purification by column chromatography on neutral Alumina (eluting with 2 – 5% MeOH/0.5% NH_4OH/CH_2Cl_2) provided **88a'** (212 mg). LCMS: LC retention time 0.55 min.; MS (ES^+) 291.2 (MH^+).

[00168] **7-Dimethylaminomethyl-1-(3-dimethylamino-propyl)-7,8-dihydro-1H-6-oxa-1,3-diaza-as-indacen-2-ylamine (89a')** was prepared from **88a'** according to the procedure described in example 3 using dimethylamine (excess) as the nucleophile for mesylate displacement and purified by reverse phase preparative HPLC to provide **89a'**. $3CH_3CO_2H$ (86 mg). LCMS: LC retention time 0.40 min.; MS (ES^+) 318.2 (MH^+).

[00169] **1-(3-Dimethylamino-propyl)-7-{[methyl-(1-methyl-1H-pyrrol-2-ylmethyl)-amino]-methyl}-7,8-dihydro-1H-6-oxa-1,3-diaza-as-indacen-2-ylamine (90ia')** was prepared from 88a' according to the procedure described in example 4 using aldehyde **17i** and purified by reverse phase preparative HPLC to provide **90ia'**. $3CH_3CO_2H$ (8 mg). LCMS: LC retention time 0.47 min.; MS (ES^+) 397.3 (MH^+).

[00170] **Example 15 – Synthesis of double constrained 2-aminobenzimidazoles (benzopyran series)**

[00171]



[00172]

[00173] tert-Butyldimethylsilylchloride (22.08 mmol, 3.33 g) was added to a cold (0 °C) solution of crude amine 23 (18.4 mmol, 2.43 g, prepared according to procedure described in example 6), Et_3N (22.08 mmol, 3.08 mL) and DMAP (catalytic) in dry CH_2Cl_2 (20 mL). After stirring at rt for 14h, the reaction was diluted with CH_2Cl_2 and the organic layer was sequentially washed with water, brine, dried ($MgSO_4$) and concentrated to provide protected amine 91, which was used without any further purification.

[00174] Amine **91** (4 mmol) was added to a suspension of diacetate 67 (4 mmol, 1.32 g) and $CaCO_3$ (4 mmol, 0.4 g) in CH_2Cl_2 (10 mL). After stirring at rt for 14 h, the reaction was diluted with CH_2Cl_2 and the organic phase was washed with water, brine, dried ($MgSO_4$) and concentrated to provide crude **92**.

[00175] A suspension of **92** (5.46 mmol, 3 g) and KOH (32.76 mmol, 1.84 g) in MeOH (55 mL) was stirred at 40°C for 48h after which the reaction mixture was evaporated to dryness under vacuum. The reaction was purified by column chromatography to provide **93** (770 mg) and **94** (714 mg).

[00176] A solution of **94** (1.58 mmol, 0.71 g) in EtOH (30 mL) was treated with 10% Pd/C (72 mg) and the mixture was hydrogenated at atmospheric pressure for 20 h to provide crude **95** which was used without any further purification.

[00177] Benzoylisothiocyanate (1.38 mmol, 0.186 mL) was added drop-wise to a cold (0°C) solution of **95** (1.38 mmol) in dry CH_2Cl_2 (3 mL). After stirring for 2h at 0 °C, the reaction was treated with diisopropylethylamine (3.1 mmol, 0.5 mL) and polymer supported dicyclohexylcarbodiimide (Resin-DCC, 4.14 mmol, 3.34 g of resin). The reaction was stirred at rt for 14h after which it was filtered through a sintered glass funnel and the resin was washed with additional CH_2Cl_2. The combined filtrates were collected and evaporated to dryness under vacuum to provide crude **96**, which was used without any further purification.

[00178] Crude **96** (1.36 mmol, 0.73 g) was refluxed in a mixture of dioxane (0.8 mL) and 10% HCl (1.86 mL) for 8 h after which an additional amount of 10% HCl (1 mL) was added to the reaction mixture. After stirring at rt for an additional 14 h, the reaction was basified with solid sodium hydroxide (ph>12), which resulted in the precipitation of pure **97** that was collected, dried and used without any further purification.

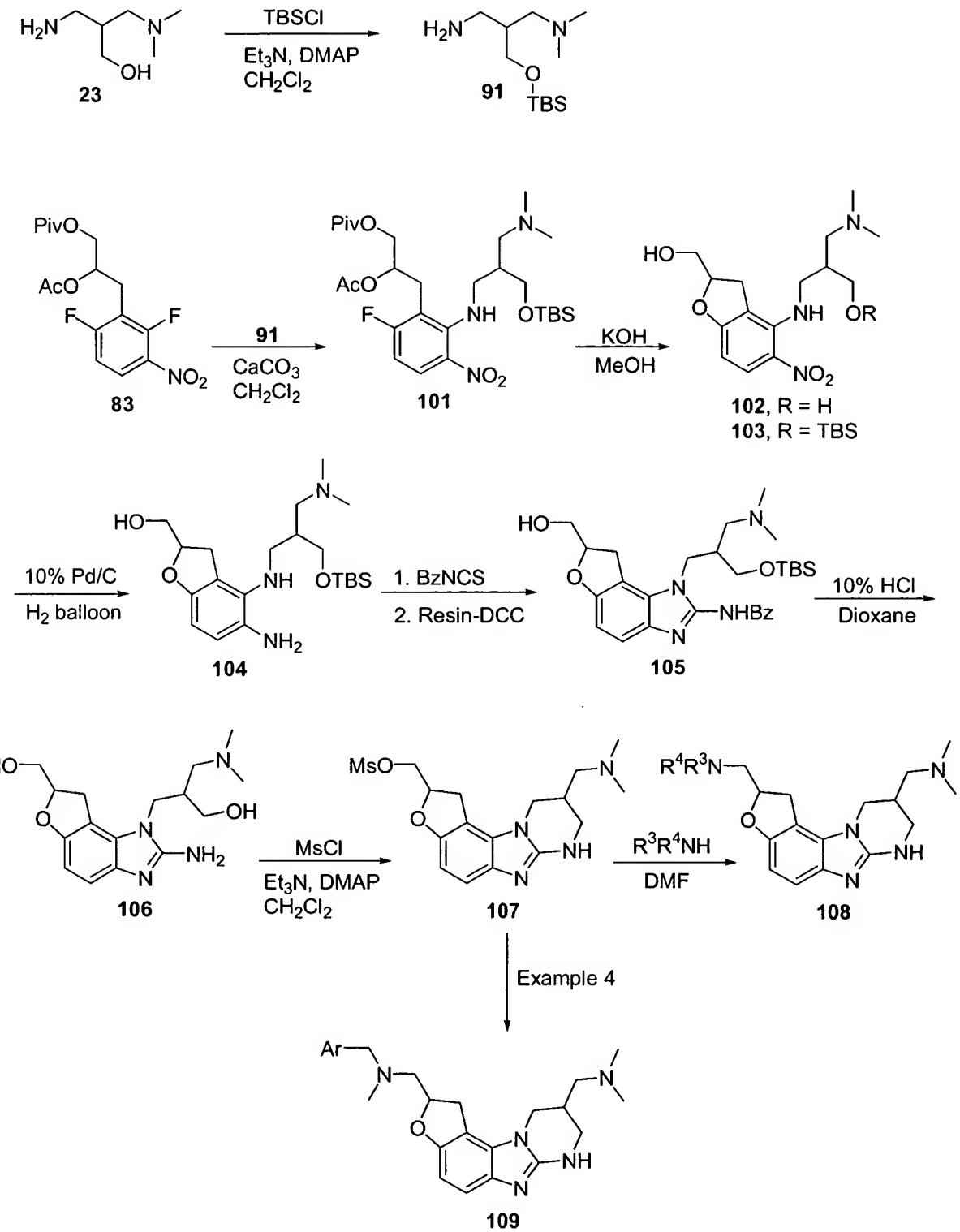
[00179] Methanesulfonyl chloride (0.96 mmol, 0.075 mL) was added to a cold (0 °C) solution of **97** (0.34 mmol, 0.117 g), triethylamine (0.96 mmol, 0.13 mL) and DMAP (catalytic) in dry CH_2Cl_2 (1 mL). After stirring for 3h, the reaction was diluted with CH_2Cl_2 and the organic phase was sequentially washed with sat. Na_2CO_3, brine, dried and concentrated to provide crude **98**, which was used without any further purification.

[00180] The desired amine (1 – 5 eq) was added to a solution of crude **98** (0.96 mmol) in DMF (0.2 mL) and the reaction was heated at 40-50 °C for 14 h. The solvent was evaporated under vacuum and the residue was purified by reverse phase preparative HPLC to provide **99**.

[00181] Benzimidazole **100** is prepared from intermediate **98** by following the general procedure outlined in example 4.

[00182] **(10-Dimethylaminomethyl-2,3,8,9,10,11-hexahydro-1H-4-oxa-7,8,11a-triaza-benzo[c]-fluoren-2-ylmethyl)-dimethyl-amine (99a')** was prepared from **98** according to the procedure described in example 15 using dimethylamine (excess) as the nucleophile for mesylate displacement and purified by reverse phase preparative HPLC to provide **99a'**. $3CH_3CO_2H$ (20 mg). LCMS: LC retention time 0.39 min.; MS (ES^+) 344.3 (MH^+).

[00183] **Example 16 – Synthesis of double constrained 2-aminobenzimidazoles (benzofuran series)**

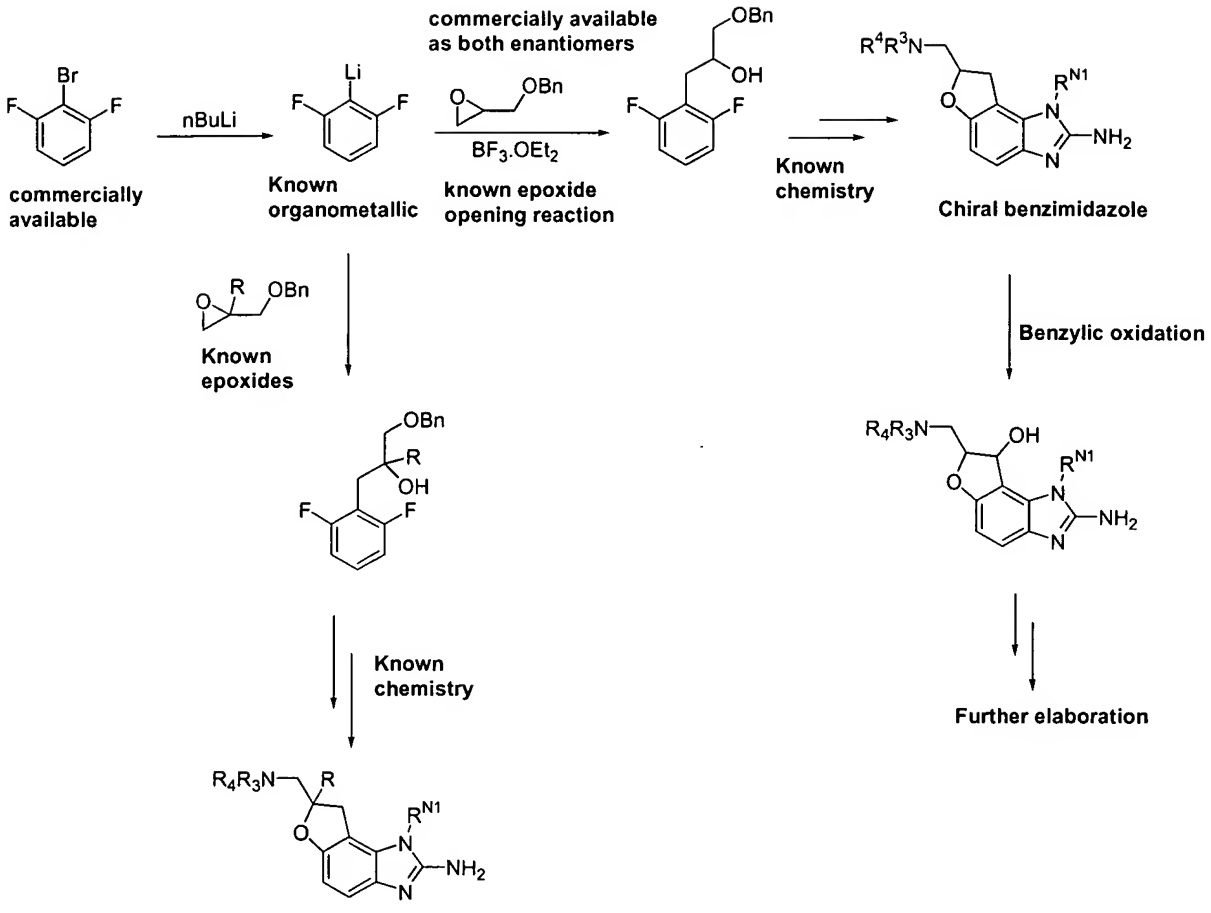


[00184]

[00185]

[00186] Benzimidazole **108** and **109** are prepared according to the general procedure outlined in example 15 except that pivaloate **83** (example 14) is used as the starting material in place of **67**.

[00187] **Example 17 – Synthesis of enantiomerically pure constrained 2-aminobenzimidazoles**



[00188]

[00189] Benzimidazoles consisting of a single enantiomer and/or diastereomer are prepared according to the general procedures used in examples 1-16, except that chirally pure synthons may be substituted for the achiral ones utilized in the previous examples. These synthons are either commercially available, known in the scientific literature, or readily prepared from known materials according to techniques known in the art. As an example, the compounds of examples 14 and 16 can be prepared using an enantiomerically enriched epoxide, which is commercially available. Subsequent elaborations as described in previous examples provide the desired compounds.

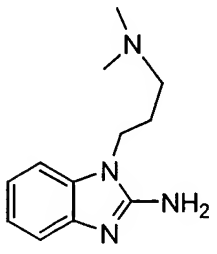
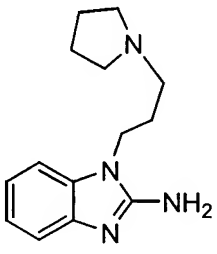
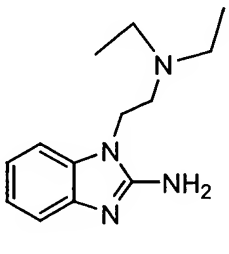
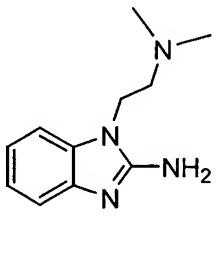
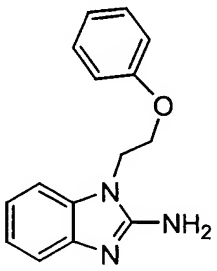
[00190] **Example 18. Mass Spectrometry Based Binding Asay.** Screening was performed by measuring the formation of non-covalent complexes between a single ligand or ligand mixture and the appropriate RNA target, along with suitable control structured RNA target(s) simultaneously using a 9.4 T FT-ICR mass spectrometer as detector. Full experimental details of the assay have been described in related literature (Sannes-Lowery, et al. in *TrAC, Trends Anal. Chem.* **2000**, *19*, 481-491 and Sannes-Lowery, et al. in *Anal. Biochem.* **2000**, *280*, 264-271. In a typical experiment, 10 μL of an aqueous solution containing 100 mM ammonium acetate buffer, 2.5 or 5 μM of each RNA; and 33% isopropyl alcohol (to aid ion desolvation) was prepared with different concentrations of each ligand or ligand mixture Samples were introduced into the electrospray ionization source (negative ionization mode) at 1 μL/min and ions were stored for 1 sec in an RF-only hexapole following desolvation. The abundances were integrated from the respective ions for free RNA and the ligand-RNA complex. The primary (1:1 RNA:ligand) and secondary (1:2 complex, if

observed). Screening was operated in two modes. In 'HTS' mode, mixtures of 8–12 compounds were screened simultaneously to determine hits, and in 'SAR' mode, K_D values were determined by titrating a single ligand through a concentration range of 0.25-25 μM. The peak ratios were measured at each concentration, then a plot of complex/free RNA versus concentration of ligand added was fitted to a second (or higher) order binding polynomial to determine the K_D. For measuring binding to the HCV IRES, RNA subdomains from 20-100 residues were prepared by commercial synthesis which assume the same structure as known in the literature of the HCV IRES and 5-UTR. These subdomains generally include a stable tetraloop (such as GAGA or UUCG), certain stabilizing base pairs (such as substituted GC and CG pairs for weaker pairs), as well as the residues in the natural sequences of HCV isolates. For the stem IIa region, these sequences include, but are not limited to (5' to 3'): CCU GUG AGG AAC UAC UGU CUU CAC GCA GAA AGC GUC UAG CCA UGG CGU UAG UAU GAG UGU CGU GCA GCC UCC AGG, GGA GGA ACU GCU GGA GAC GCG CAG CCU CC, GGA GGA ACU ACU GGA GAC GUG CAG CCU CC, GGA GGA ACU AGC GAG AGC UGC AGC CUC C, and GAG GAA CUA CUG UCU UCA CGC ACC GAG AGG UGA GUG UCG UGC AGC CUC.

[00191] More particular presentation of the stem region IIa of the 5'-UTR of HCV RNA can be more fully understood according to the following literature references: Kieft, J. S.; Zhou, K.; Jubin, R.; Murray, M. G.; Lau, J. Y.; Doudna, J. A. *J. Mol. Biol.* **1999**, *292*, 513-529; Honda, M.; Beard, M. R.; Ping, L. H.; Lemon, S. M. *J. Virol.* **1999**, *73*, 1165-1174; Zhao, W. D.; Wimmer, E. *J. Virol.* **2001**, *75*, 3719-3730, particularly the residues encompassed by the grey shaded area of Figure 2 as found therein. The stem IIa region of the 5'-UTR of HCV RNA can also be understood as a structure formed by residues 52-65 and residues 102-111 of the HCV RNA (Genbank accession number NC_004102, and naturally occurring variations thereof.

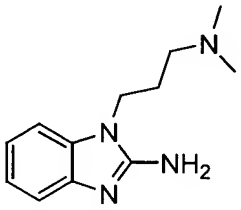
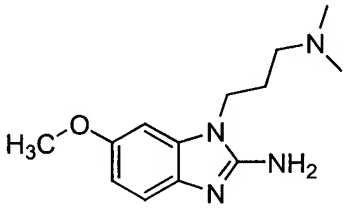
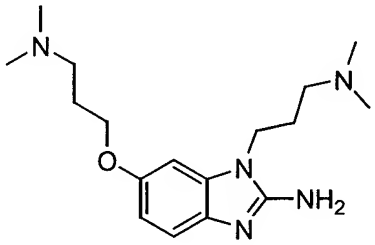
[00192] In some embodiments, compounds according to the invention may be prepared according to the following discussion. In general, compounds according to the present invention selectively bind to HCV IRES. Structure-Activity Relationships (SAR) for a class of compounds may be determined by measuring the K_D for various compounds within the class having a variety of structural features. There are some general rules that guide the artisan in determining whether an active compound is selectively active enough to be considered a candidate for further development (candidate). In this context, it should be recognized that compound that does not qualify as a candidate, nonetheless may have utility as either a positive or negative control in an assay, or may qualify as an assay standard, etc. However, a candidate will generally have an estimated K_D on the order of about 100 μM, will be at least about 4 fold more selective for the target than for non-targets, will generally demonstrate single-site binding, and will be amenable to SAR as a class. For example, for the following Table 1, the target compound (ligand, 50 μM) and IIa target (2.5 μM) were incubated to give the indicated binding percentages of ligand to target.

TABLE 1

				
56% binding	**15%**	**15%**	**11%**	**6%**

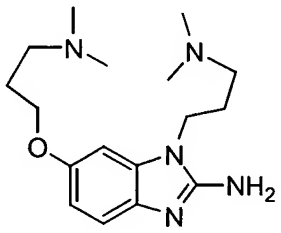
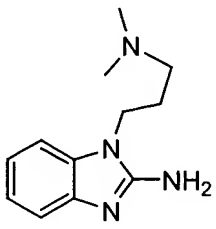
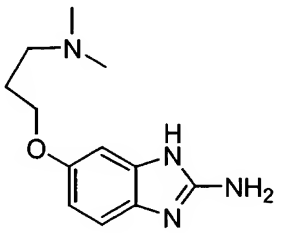
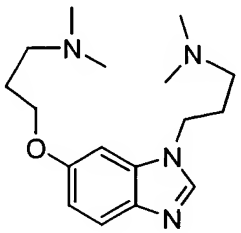
[00193] The following Table 2 shows the progression in binding affinity and MS target selectivity for some modifications on the 2-aminobenzimidazole ring.

TABLE 2

			
Est. K_D (μM)	~ 100	~ 40	~ 10
MS target selectivity	~ 3	~ 10	~ 15

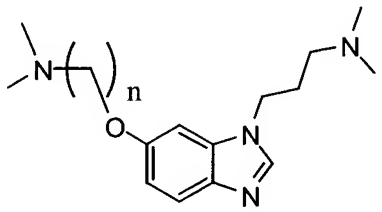
[00194] As can be seen in the foregoing Table 2, the selected modifications resulted in an approximately 10-fold improvement in binding affinity and about a 5-fold improvement in target selectivity across the series. As can be seen from the following Table 3, each of the N1, 2- and 6- position substituents are critical for binding to HCV IRES.

TABLE 3

				
Est. K_D (μM)	~ 10	~ 100	> 500	>500
MS Target Selectivity	~ 15	~ 3	~ 2	1

[00195] SAR was performed on the C6-alkyl "tether" (i.e. the alkylene moiety connecting O6 to the amine group. The results for the compounds below are shown in Table 4:

TABLE 4

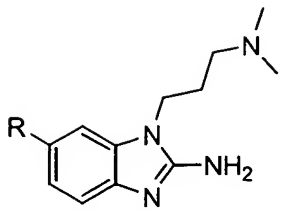


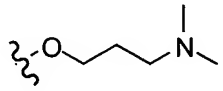
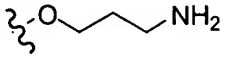
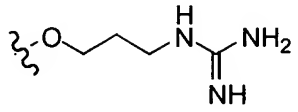
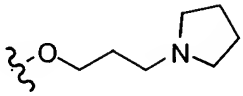
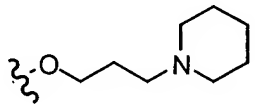
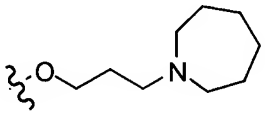
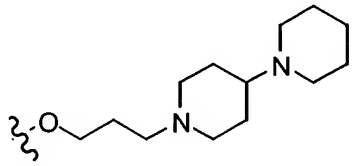
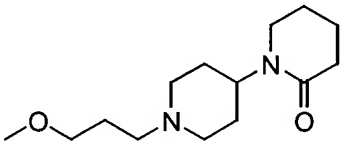
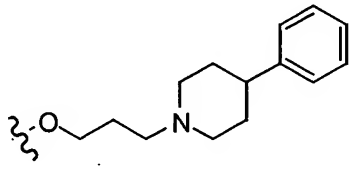
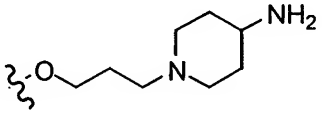
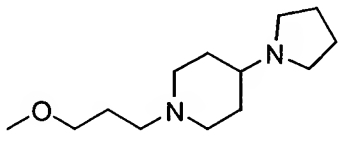
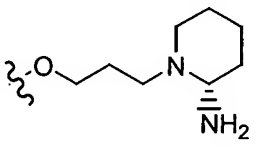
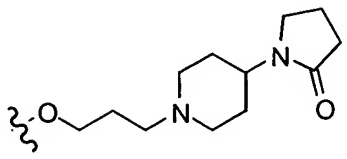
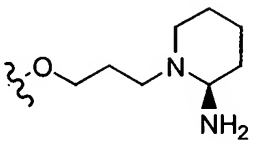
n	K_D (μM)	Target Selectivity
2	7	7
3	9	15
4	8	11

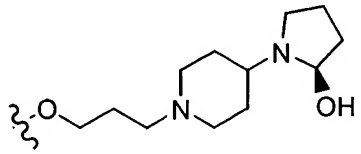
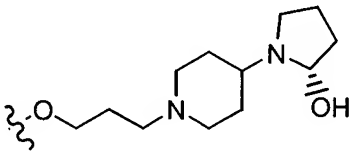
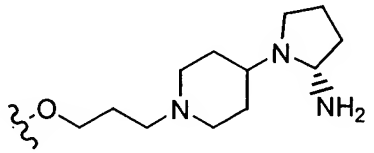
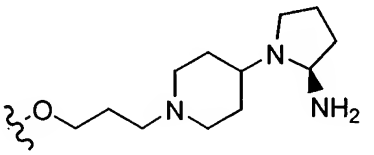
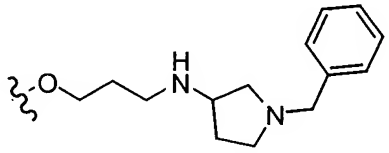
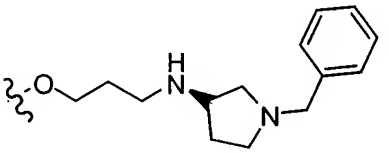
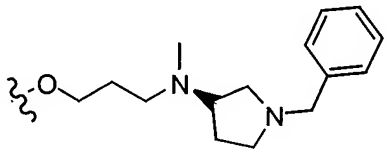
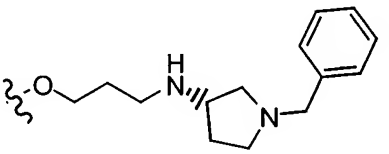
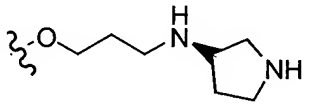
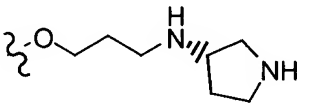
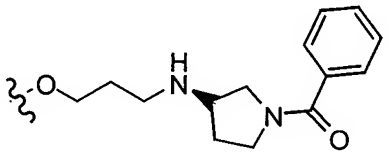
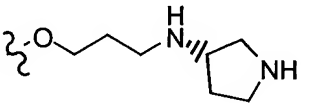
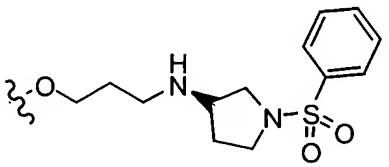
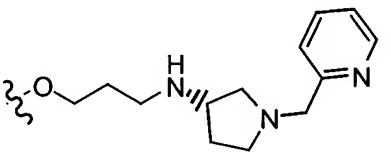
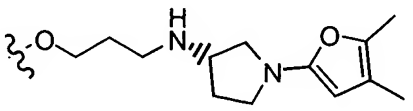
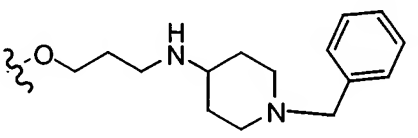
[00196] As can be seen above, the K_D at this site is relatively insensitive to tether length, but target selectivity can be improved about 2-fold by selecting n = 3 versus n = 2.

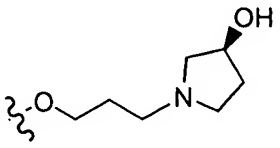
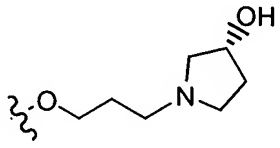
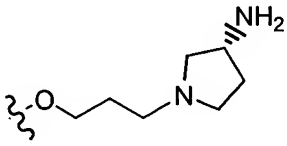
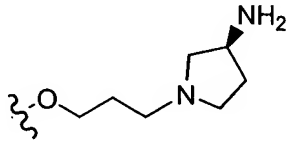
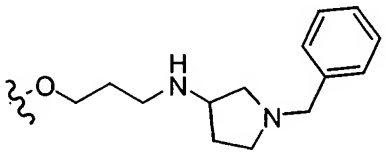
[00197] In the following Table 5, various C-6 side chain modifications are presented, along with their K_D values.

TABLE 5



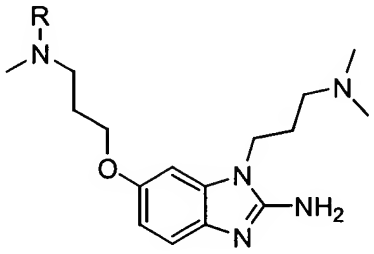
R	K_D (μM)	R	K_D (μM)
	3.6		2.3
	4.6		3.4
	3.7		7.1
	0.71		2.2
	5.5		0.66
	0.78		0.44
	2.6		0.74

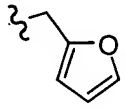
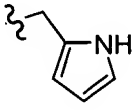
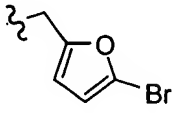
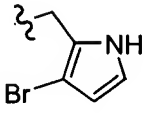
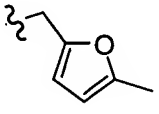
R	K_D (μM)	R	K_D (μM)
	2.7		2.3
	0.78		0.83
	1.0		1.9
	3.1		2.2
	0.49		2.6
	2.2		0.66
	4.5		2.3
	1.8		0.51

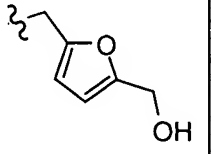
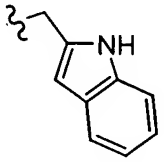
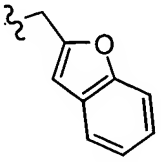
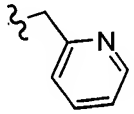
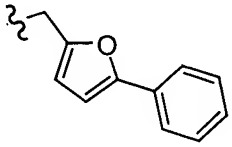
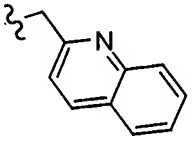
R	K_D (μM)	R	K_D (μM)
	2.7		2.3
	0.78		0.83
	1.0		

[00198] Further SAR was performed at the 6-Position on the N- of the (4-aminobutoxy) group. The results of this SAR are shown in Table 6 below:

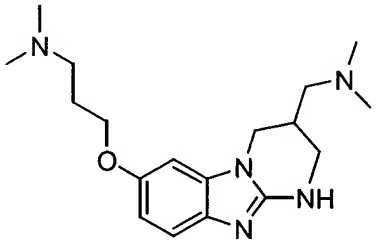
TABLE 6



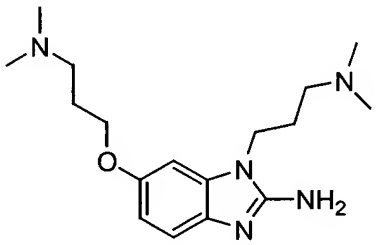
R	K_D (μM)	R	K_D (μM)	R	K_D (μM)
	3.2		2.1		2.1
	5.6		5.8		4.4

	5.4		8.5		6.5
	4.4		6.5		21

[00199] In order to probe the effect of constraining the N1 and N2 side chains, the constrained compound:

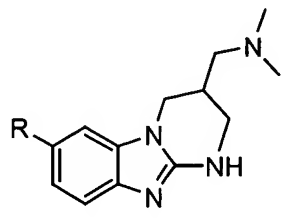
[00200]  ,

[00201] which had a K_D of 0.67 μM, as compared to a K_D of 3.6 μM for the unconstrained compound:

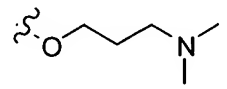
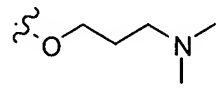
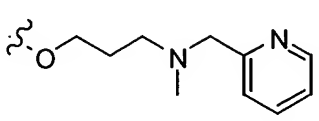
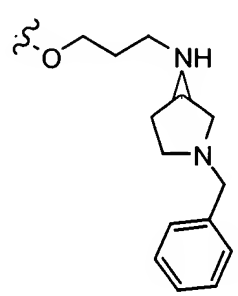
[00202]  .

[00203] It would appear from the foregoing observation that the constrained N2 side chain has favorable binding characteristics. Accordingly, it was hypothesized that N1-N2 constrained compounds could be identified having improved properties by probing the SAR of 6-position substitutions having a shared constrained core. The results of this study are presented in Table 7 below:

TABLE 7



	R	K_D (μM)	R	K_D (μM)

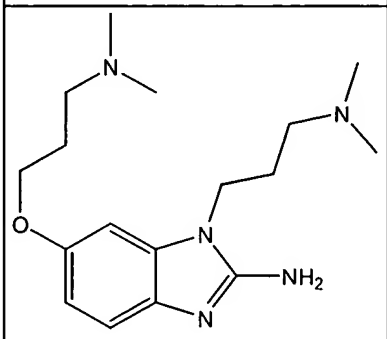
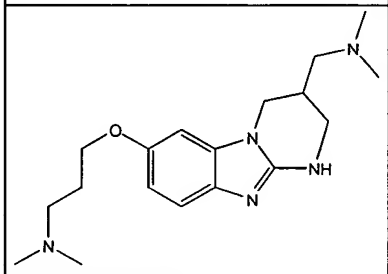
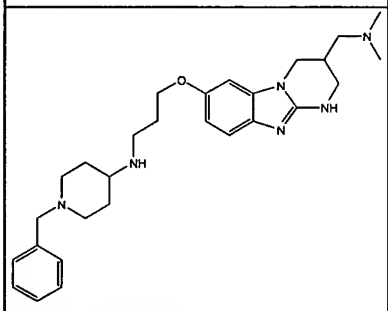
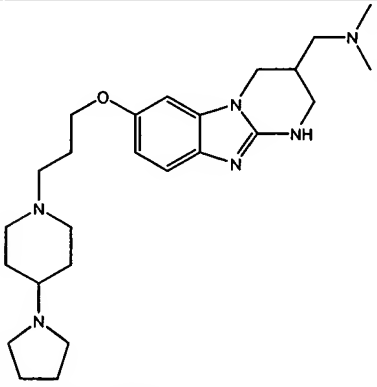
	0.67		0.35
	0.43		0.53

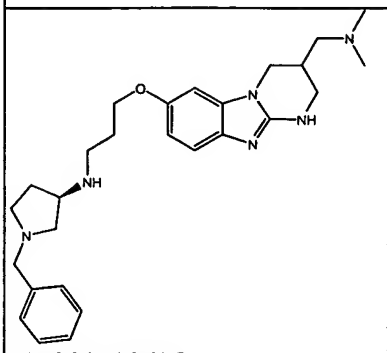
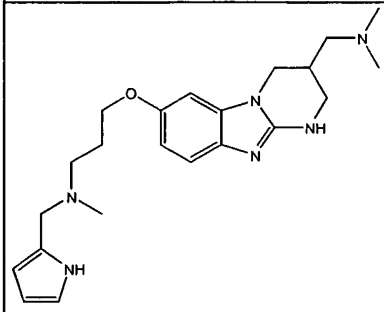
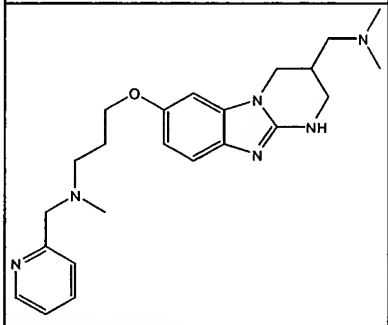
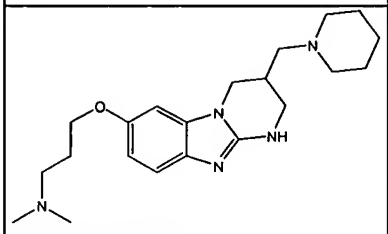
[00204] **Example 19 Replicon Assay**: The Ntat2ANeo replicon containing cell line was obtained from Dr. S. Lemon at the University of Galvaston. Cells were grown, handled, treated with compound, and evaluated for HCV RNA levels as described previously (Yi, M.; Bodola, F.; Lemon, S. M. *Virology* **2002**, *304*, 197-210.) Briefly, the Ntat2ANeo cells were seeded into 96-well plates. The media was replaced 24 h later with fresh, G418-free media containing the indicated concentrations of drug. After the appropriate incubation period, cells were harvested, and quantitative RT-PCR assays were carried out using TaqMan chemistry on a PRISM 7700 instrument (ABI). For detection and quantitation of HCV RNA, primers complementary to the 5'-NTR region of HCV (Takeuchi, T., Katsume, A., Tanaka, T., Abe, A., Inoue, K., Tsukiyama-Kohara, K., Kawaguchi, R., Tanaka, S., and Kohara, M. *Gastroenterology* **1999**, *116*, 636–642.) were used. Results were normalized to the estimated total RNA content of the sample, as determined by the abundance of cellular GAPDH mRNA detected in a similar real-time RT-PCR assay using reagents provided with TaqMan GAPDH Control Reagents (Human) (Applied Biosystems).

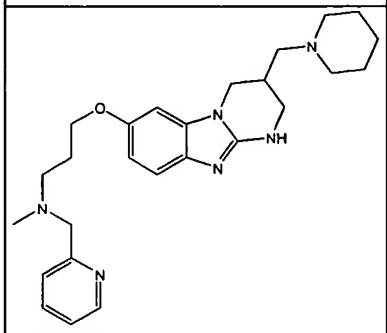
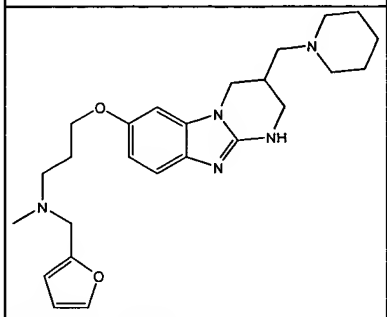
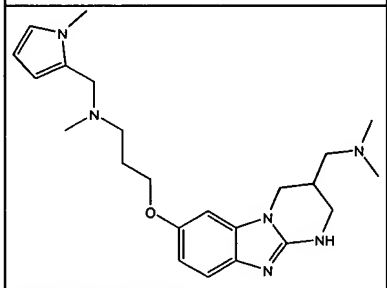
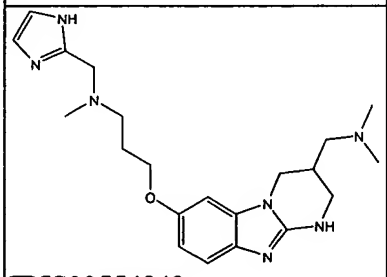
[00205] **Example 20 MTT Toxicity Assay**: The MTT cell proliferation assay was used to test our compounds for cell toxicity (v van de Loosdrecht, A. A.; Beelen, R. H.; Ossenkoppele, G. J.; Broekhoven, M. G.; Langenhuijsen, M. M. *J. Immunol. Methods* **1994**, *174*, 311-320. The assay kit was purchased from American Type Culture Collection (Manassas, VA, USA), and treatment of cells and the specific assay protocol was carried out according to the manufacturer's recommendations. The MTT cell proliferation assay measures cell viability and growth by the reduction of tetrazolium salts. The yellow tetrazolium salt is reduced in metabolically active cells to form purple formazan crystals which are solubilized by the addition of detergent. The color was quantified by spectrophotometric means. For each cell type a linear relationship between cell number and absorbance is established, enabling quantification of changes of proliferation.

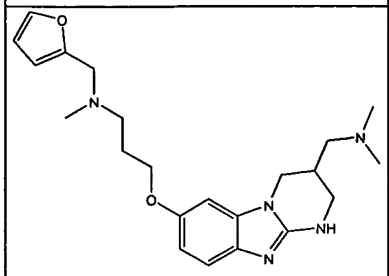
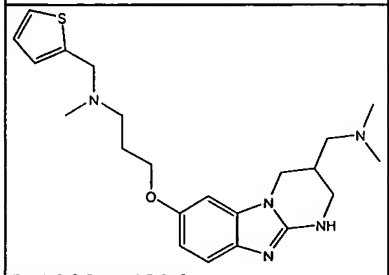
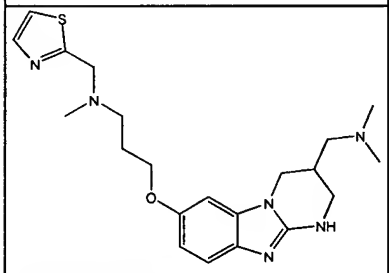
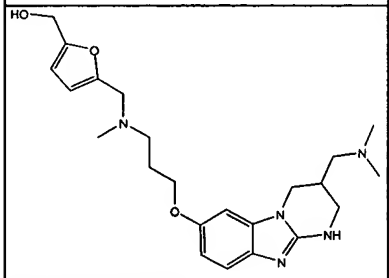
[00206] Compounds according to the present invention were subjected to a replicon assay as described in example 19, as well as an MMT assay as described in example 20. Advantageous compounds according to embodiments of the present invention include, but are not limited to, those listed in Table 8.

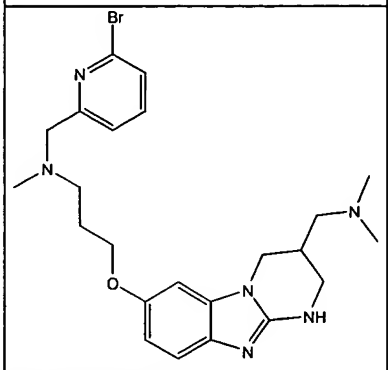
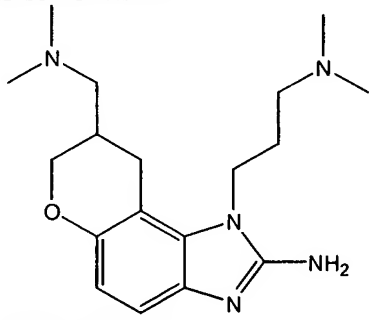
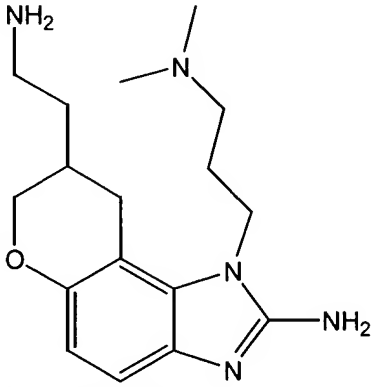
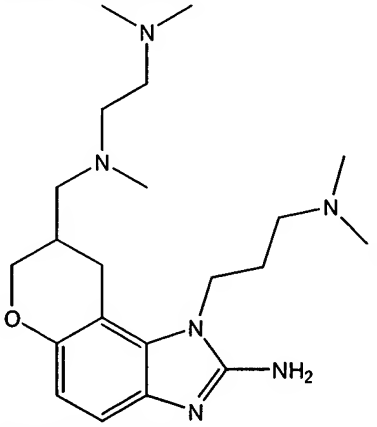
TABLE 8

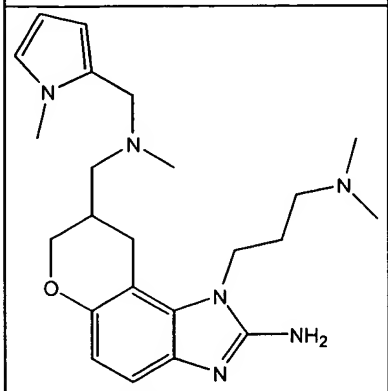
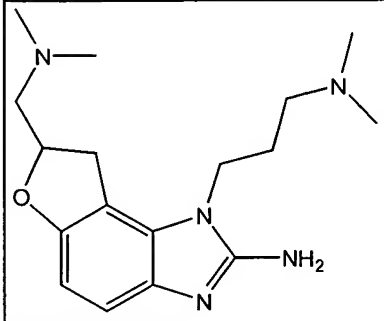
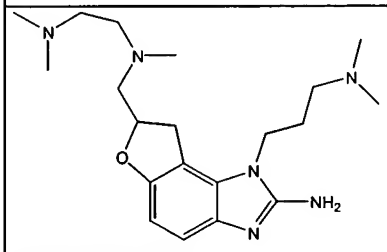
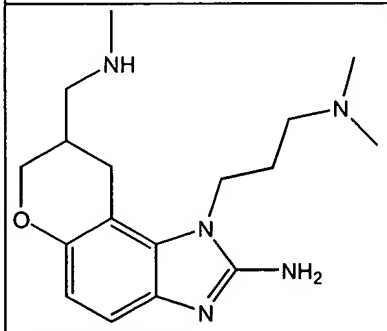
Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 **IBIS00403514**	3.8	37.1	
 **IBIS00408169**	**4.5**	**28.7**	**>100 (48)**
 **IBIS00528633**	**4.0**	**13.7**	**>100 (48)**
 **IBIS00528634**	**1.7**	**19.2**	**>100 (48)**

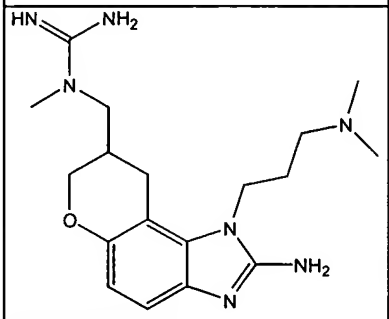
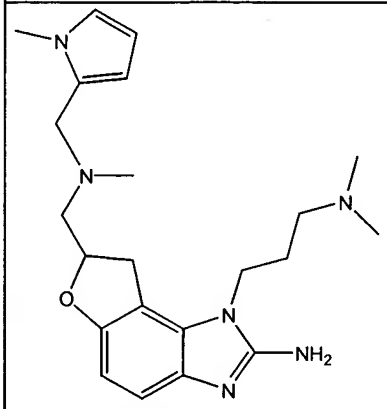
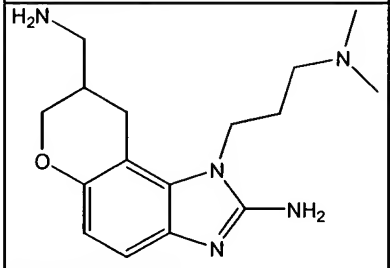
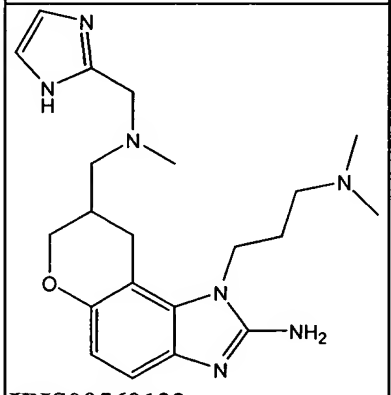
Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 IBIS00528635	1	42.3	90 (48)
 IBIS00528636	1.2	27.2	90 (48)
 IBIS00528637	0.56	14.2	>100 (48)
 IBIS00554807	3.4	14.1	>100 (48)

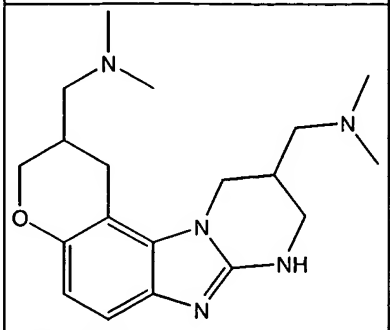
Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 IBIS00554811	4.3	24.0	>100 (48)
 IBIS00554813	5.1	18.2	>100 (48)
 IBIS00554842	0.78	4.0	50 (48)
 IBIS00554843	3.1	> 100	>100 (48)

Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 IBIS00554844	1.7	4.9	>100 (48)
 IBIS00554886	5	15.3	>100 (48)
 IBIS00554888	8.8	> 100	>100 (48)
 IBIS00554889	5.9	57.9	>100 (48)

Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 **IBIS00560002**	**110**	**11.6**	**>100 (48)**
 **IBIS00560020**	**2.2**	**9.0**	**>100 (48)**
 **IBIS00560024**	**3.4**	**> 100**	**>100 (48)**
 **IBIS00560025**	**9.4**	**71.2**	**>100 (48)**

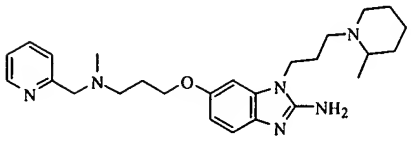
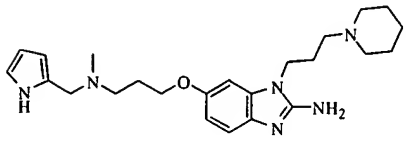
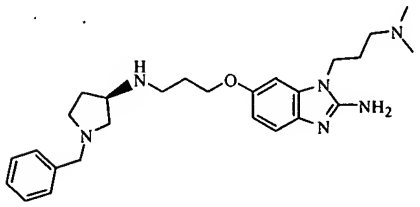
Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 **IBIS00560031**	**18**	**21.7**	**>100 (48)**
 **IBIS00560047**	**2.1**	**1.5**	**>100 (48)**
 **IBIS00560048**	**11**	**23.0**	**>100 (48)**
 **IBIS00560100**	**3.1**	**51.6**	**>100 (48)**

Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 **IBIS00560101**	**5.5**	**> 100**	**>100 (48)**
 **IBIS00560102**	**10.4**	**3.3**	**>100 (48)**
 **IBIS00560121**	**17.2**	**42.7**	**>100 (48)**
 **IBIS00560122**	**19.6**	**> 100**	**>100 (48)**

Compound	Kd to IIa target (μM)	Replicon IC_{50} (μM)	MTT CC_{50} (μM); time (h)
 IBIS00560146	21.1	5.4	>100 (48)

[00207] **Example 21 - Acute *in vivo* Toxicity Study**. A single dose toxicity study was performed to investigate the toxicity of representative compounds. Briefly, 3-4 female mice per group were administered 0, 5, or 45 mg/kg drug (intraperitoneal) on consecutive days for 3 days. At the end of the study, mice were sacrificed, and clinical signs, body weights, clinical pathology, organ weights, and histopathology endpoints were evaluated. Representative compounds were found to exert no obvious toxic effects at pharmacologically relevant doses. The data is summarized in Table 9. The gross findings at necropsy were minor and limited to discolorations in liver and kidney or large gallbladder in 4/18 mice (no dose response and unclear relationship to drug). The organ weights showed no effects. There were no drug-related effects on clinical pathology.

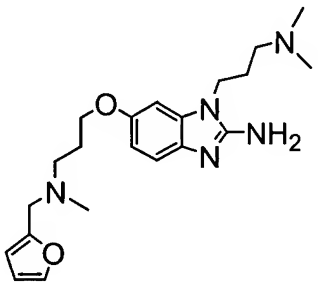
TABLE 9

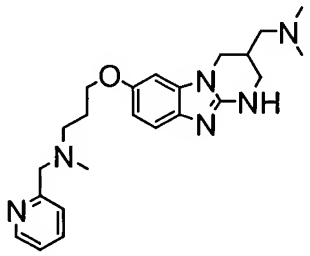
Structure	Ibis Number	Clinical Signs
 ·3HCl	IBIS00553642	No significant toxicity at 5 and 45 mg/kg doses.
 ·3HCl	IBIS00408094	No significant toxicity at 5 and 45 mg/kg doses.
 ·4HCl	IBIS00405746	No significant toxicity at 5 mg/kg dose.

[00208] **Example 22 - Single Dose *in vivo* Pharmacokinetic Study**. A single dose pharmacokinetic study was performed to investigate the pharmacokinetics of representative compounds. Of particular interest was

the ability of the compounds to accumulate in liver, which is the target tissue for HCV chemotherapy as it is the primary reservoir of virus. 3-4 rats per group were administered a single dose (3 mg/kg intravenously, IV or 6 mg/kg orally, PO). Blood samples were taken at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h timepoints, and pharmacokinetic parameters calculated. The data is summarized in Table 10. Both representative compounds were rapidly distributed to tissues, as evidenced by the high clearance rates and volumes of distribution, and low levels of excretion (ca 10% of total drug in urine and feces at 8 h). Major tissues were examined for presence of drug. Liver, kidney, and lung showed the highest concentration, with liver concentrations achieving ca 8 μg/g tissue 8 h after a single 3 mg/kg IV dose. The tissue accumulation for the IV dose is shown in Figure 1, as a function of concentration and percent of total administered dose. Oral bioavailability was also studied, and both compounds showed ca 25% oral plasma bioavailability. The plasma concentration vs. time profile for both IV and PO dosing routes for a representative compound is shown in Figure 2. This data provides that the compounds described herein are present in target tissues, including liver, following oral administration to show an antiviral effect *in vivo*.

TABLE 10





		IBIS00405678		IBIS00528637	
Parameter	Units	Mean	%CV	Mean	%CV
Original Dose	mg/kg	3.0		3.0	
AUC	ng*Hours/ml	311	21.3	165	2.9
AUC Extrap	ng*Hours/ml	1928	51.2	320	19.0
Co	ng/ml	201	11.4	219	10.0
T1/2	Hours	37.5	55.4	14	43.8
MRT	Hours	3.1	5.8	2	5.6
CL	mL/hr/kg	2069	72.6	9585	17.1
Vdss	mL/kg	82806	22.9	136706	29.5
% AUC Extrap	%	80.0	14.6	47	21.8

[00209] Each of the patents, applications, and printed publications, including books, mentioned in this patent document is hereby incorporated by reference in its entirety. This application further relates to provisional serial number 60/429595 filed 11/26/02, now PCT application bearing the same title and is incorporated herein by reference in its entirety.

[00210] As those skilled in the art will appreciate, numerous changes and modifications may be made to the preferred embodiments of the invention without departing from the spirit of the invention. It is intended that all such variations fall within the scope of the invention.